Stony Brook University

Synthetic Studies on Difluorovinyl-Taxoid Anticancer Agents and Benzimidazole-Based Antituberculosis Agents

A Thesis Presented

By

Guan-Ting Chen

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Abstract of the Thesis

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In the effort towards anti-cancer and infectious disease agents discovery, two classes of compounds were developed by the Ojima group. One is taxoids based anti-cancer targeting agents, the other is benzimidazole anti-TB agents.

In the past, the Ojima group was able to prepare enantiopure β-lactam through [2+2] ketene-imine cycloaddition followed by the enzymatic optical resolution of racemic β-lactam. Although, the enzymatic resolution method allows high enantiopurity, it can only give maximum of 50% yield from the racemic-β-lactam and can be time consuming. For optimization, an alternative synthesis was proposed with the use of a naturally occurring, less expensive and commercially available starting material- D-mannitol. With the new proposal, stereoselective designed difluorovinyl β-lactam was synthesized within 11 steps in higher yield and was used for beccatin based second generation anticancer reagent.

A series of 2,5,6-trisubstituted benzimidazole intermediates were synthesized for library synthesis in 96-well plate. These included, 5,6-diamine-*N*,*N*-diethyl-2-cyclohexyl benzimidazole, 5,6-diamine-*N*,*N*-diethyl-2-(4-methoxyphenyl)-benzimidazole, and 5,6-diamine-*N*,*N*-diethyl-2-(2-methoxyphenyl)-benzimidazole. SB-P8G2, (2-cyclohexyl-6-pyrrolidin-benzimidazol-butylcarbamate) as part of the 2,5,6-trisubstituted benzimidazole series were synthesized for *in vitro* and *in vivo* studies. The synthetic methods of each step (total of 5 steps) were improved in the means of reacting conditions, purifications, as well as chemoselectivity.

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Chapter I

Synthetic Studies on Difluorovinyl-Taxoid Anticancer Agents

1.1. Introduction

1.1. 1 Therapeutic agent targeting tubulin

Cancer has proven to be one of the most deadly diseases worldwide due to the uncontrollable proliferation of cells, diminished apoptosis, invasion and metastasis. It is the second leading cause of death in the United Sates and has surpassed heart disease to become the number one cause of death for those under the age of 85 according to the American Cancer Society. From the statistics, it is concluded that one in two men and one in three women living in North America will develop some form of cancer in their life time (1).

While it is not fully understood, it is believed that cancer could be caused by either external or internal factors, which mutates genes that encode proteins controlling cell division. External factors are carcinogens which includes exposure to chemicals, radiation and viruses. Internal factors are genetic related abnormalities such as change in hormones, immune conditions or inherited mutations. Common causes of cancer are smoking, obesity, physical inactivity and infections.

Unlike normal cells that divides and dies, the accelerated, uncontrolled growth of cancer cells results in a mass, which is called a tumor. Tumors are either benign or malignant. A benign tumor is usually harmless, especially if it is removed before it presses against any vital organs in the body. It usually grows slowly and is covered by a membrane that keeps it separated from the normal cells around it. A malignant tumor on the contrary grows much faster and is almost never encapsulated, or covered by a membrane. Malignant cancer cells can metastasize or spread and invade other parts of the body by travelling through the bloodstream or lymph system.

General treatments for cancer patients include surgery, radiation therapy, proton therapy, bone marrow transplants, hormonal therapy, immunotherapy and chemotherapy. The choices of specific treatments are based on the location and status of the tumor, the stage of the disease as well as the condition of the patient. Of all, chemotherapy is one of the most promising cancer treatments, using potent cytotoxic drugs to destroy rapidly growing cancer cells and control or eliminate tumor growth (2) (3). Depending on properties like water solubility of the drug as an example, chemotherapeutic agents can be orally or intravenously delivered into human body.

The mechanism of action of the most cytotoxic drugs is based on the premise that cancer cells have abnormally rapid proliferating abilities compared to healthy cells, which the drug is able to distinguish and destroy. Unfortunately, the damage of non-malignant cells leads to number of undesirable side effects and makes current cancer drugs toxic to the human body. The side effects patients experience due to the lack of target specificity include hair loss, nausea and vomiting, fatigue, diarrhea or constipation, anemia, depression of the immune system, and hemorrhage (4) (5).

1.1.2 Paclitaxel: anti-cancer drug

In 1958, the National Cancer Institute (NCI) recognized the need for the development of new drugs and therapeutic procedures to treat cancerous disease and initiated a program to screen 35,000 plant species for anticancer activity (6). In 1966, crude extracts taken from the bark of the pacific yew tree (*Taxus brevifolia* Nutt) displayed tumor inhibitory properties and potent cytotoxicity against mouse leukemia. Later, the active component of the extract was isolated and characterized in 1971 by Wani and co-workers, which was identified as paclitaxel (Taxol®) (7). As of today, paclitaxel is one of the leading drugs in the taxoids family that exhibit significant antitumor activity against various cancer cell lines that are not effectively treated by other existing chemotherapeutic drugs (8).

The unique structure of paclitaxel includes a complex diterpene serving as the core which consists of four rings (A-D) and an *N*-benzoylphenylisoserine side chain at the C-13 position. Furthermore, the complexity of the molecule increases with its existing 11 chiral centers and 14 oxygen functionalities (**Figure 1-1**).

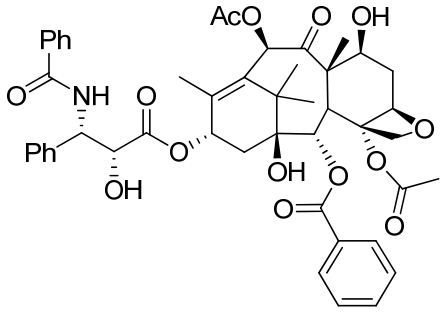


Figure 1-1: Structure of paclitaxel (Taxol®)

Paclitaxel is a mitosis inhibitor, as it is able to interfere the normal function of microtubule breakdown and inhibit cell division cycle between metaphase and anaphase. Microtubules are major components of the cytoskeleton which are also involved in many cellular processes within cells. They are formed by the dimerization of α- and β-tubulin protein subunits, which are proteins of approximately 440 amino acid residues with a molecular weight of about 50 kD each (9). In the presence of magnesium ions, guanosine 5'-triphosphate (GTP), and microtubule-associated proteins (MAPs), α- and β–tubulins form dumbbell-shaped heterodimers. These tubulin heterodimers join end-to-end and form protofilaments, which associate laterally to form sheets and eventually microtubules. The microtubule tends to be composed of 13 protofilmaents with an average diameter of about 24 nm (9).

What sets paclitaxel apart from other chemotherapeutic drugs is the fact that instead of inhibiting the tubulin polymerization, it actually promotes it. Therefore, paclitaxel enhances the rate, extent and nucleation phase of tubulin polymerization, and is known as microtubule stabilizers. This stabilization prevents dissociation or depolymerization. Under this condition, the normal dynamic reorganization of the microtubule network required for mitosis is inhibited and the signaling at G2/M stage of mitosis eventually induces apoptosis.

Taxol® is used to treat various cancers including breast cancer, ovarian cancer, lung cancer, head and neck cancer, bladder cancer and AIDS-related Kaposi's sarcoma. Although Taxol® is considered one of the most chemotherapeutic drugs there are still undesirable issues which are of concerns. One of them is the lack of specificity. Due to the little difference in the drug activity against tumor tissues and healthy tissues, a variety of undesirable side effects can occur. These include allergic reactions, infections due to low white blood cell count, hair loss, joint and muscle pain, diarrhea and neuropathy. The drug is also subject to multi-drug resistance (MDR) and it is not water-soluble (9). Improvement needs to be made on the activity, efficacy, specificity and toxicity of Taxol® and related chemotherapeutic agents.

One of the original problems with Taxol® production is the scarcity of the Pacific yew. The bark of the Pacific Yew tree is a nonrenewable resource and extraction process was cumbersome and low-yielding. It required 10,000 kg of the bark to obtain 1 kg of

paclitaxel (0.01 % yield). This problem has been solved as scientists have developed a semi-synthetic means to produce paclitaxel and its analogs from 10-deacetylbaccatin III (10-DAB III) (**Figure 1-2**), which is isolated from the leaves of the European yew (*Taxus baccata*). Unlike the bark of Pacific yew, the leaves of European yew are a renewable source and the isolation is achieved in good yields. Accordingly, this semi-synthesis method, the coupling of the C-13 side chain to a modified 10-DAB III core, is a great approach that ensures a long-term supply of paclitaxel.

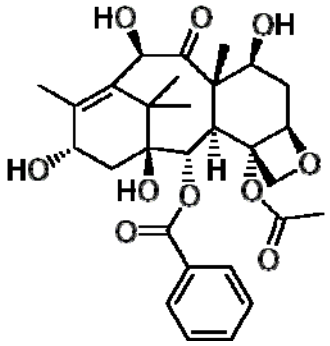


Figure 1-2: 10-Deacetylbaccatin III (10-DAB III)

For a more practical and efficient semi-synthesis of paclitaxel, Ojima *et al*, introduced the β-Lactam Synthon Method using an enantiopure β-lactam as the key structural skeleton and synthetic intermediate (10). This β–lactam was used as the precursor of the paclitaxel side-chain. Modifications of this β-lactam can be used to produce different taxanes and taxane analogs (11).

β-lactams have a heteroatomic ring structure, consisting of three carton atoms and one nitrogen atom and is a part of the structure of several antibiotic families including penicillins. These bactericidals act by inhibiting the synthesis of the peptidoglycan layer of the bacterial cell walls. The inhibition can be lethal due to its importance in cell wall structural integrity, especially in Gram-positive organisms (12). Under normal conditions, the synthesis of peptidoglycan is completed by the final transpeptidation step facilitated by penicillin-binding proteins (PBPs), transpeptidases. The PBPs recognize the terminal amino acid residues, D-alanyl-D-alanine, on the precursor peptide subunits of the nascent peptidoglycan layer and facilitate the final cross-linking process. However, when β-lactams, analogues of D-alanyl-D-alanine, are introduced into the system, its structural similarity allows it to interact with PBPs and disrupt the cell wall synthesis in an

irreversible manner. The inhibition of cross-linkage by β-lactams eventually leads to the build-up of peptidoglycan precursors, which further leads to the digestion of existing peptidoglycan (12).

The synthesis of β–lactam was first introduced by Staudinger in 1907. The reaction involves the addition of a ketene and an imine, in which both reactants can act as the electrophile or nucleophile to form the four-member ring structure. This reaction was later known as a [2+2] cycloaddition after the advent of the Woodward-Hoffman rules. At present, a modified Staudinger reaction is used in the Ojima group for the synthesis of enantiopure β–lactams. The reaction involves the treatment of an imine with an acyl chloride in the presence of base. The lactam ring forms as the acyl chloride cyclocondenses with the imine (13). The stereoselectivity was well controlled by reducing temperature. The specific enantiomer of β-lactam was resolved by an enzymatic resolution (14). By coupling the synthesized β-lactam with 10-DAB III derivative, the taxoid analogs, SB-T-1214 (**Figure 1-3**), SB-T-1216 (**Figure 1-4**) and SB-T-12854 (**Figure 1-5**) had been produced. These new-generation taxoids exhibit substantially higher potency than that of paclitaxel against drug-resistant cell lines expressing MDR phenotypes (15).

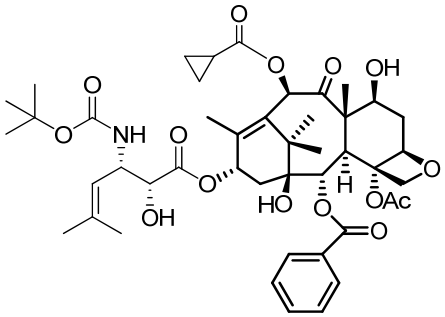


Figure 1-3: Structure of SB-T-1214

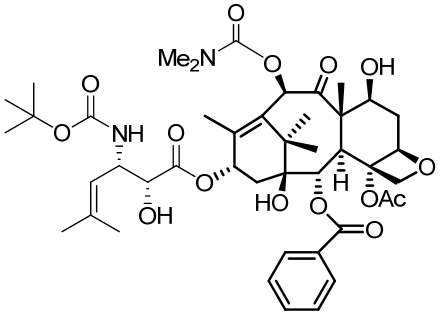


Figure 1-4: Structure of SB-T-1216

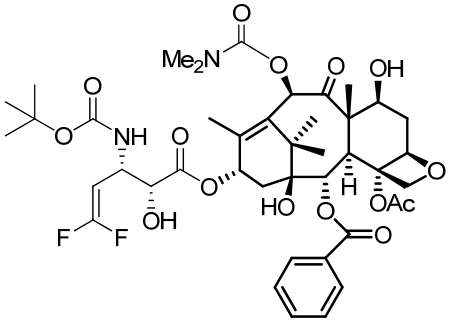


Figure 1-5: Structure of SB-T-12854

1.1.3 Synthesis of β-Lactam

In the presence of a tertiary amine, acetoxyacetyl chloride cyclocondenses with an imine to give a racemic cis-*β*-lactam, (±)-1-(4-methoxyphenyl)-3-acetoxyl-4-(2-methylprop-1-enyl)azetidin-2-one, **1-2**. The [2+2] ketene-imine cycloaddition (16) was known as the Staudinger reaction (17). It is one of the most important and direct approaches for the stereoselective construction of β-lactam skeleton from readily available Schiff bases and ketenes (18) (19). The reaction between a ketene and an imines result in [2+2] cycloaddition (20). Typically, acyl chlorides can be converted to ketene by treatment of a base, followed by reaction with an imine to form a zwitterionic intermediate. The reaction then undergoes an electrocyclic conrotatory ring closure to give the β-lactam ring with the cis conformation (**Figure 1-6**).

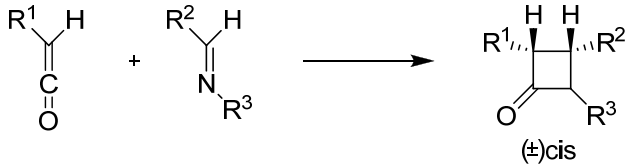


Figure 1-6: Synthesis of β-lactams by Staudinger reaction

1.1.4 Synthesis of Difluorovinyl second-generation taxoids

Taxol® has excellent anticancer activity, but it has a number of undesirable side effects as well as inherent weakness against drug-resistant cancer cells expressing multi-drug resistance (MDR) phenotypes. To overcome these shortcomings, development of new analogs is necessary to maximize the capability of the taxane-based anticancer agents.

Since the basic principles for modification of biological activity of organic compounds via fluorine substitution of hydrogen was established, the fluorine-containing biologically active molecules became very attractive synthetic target. Fluorine is a unique atom. It is the second smallest atom after hydrogen with van der Waals radius of 1.35 Å and the most electronegative element (3.98). The van der Waals radius of fluorine is larger than hydrogen atom but smaller than a lot of functional groups such as methyl, amino or hydroxyl group. Hence, the substitution of hydrogen with fluorine in the molecule introduces minimal steric alterations; with very high electronegativity, fluorine can alter density, acidity, and basicity of neighboring groups, changing physical,

chemical properties of the molecule. The carbon-fluorine bond is at least 14 Kcal/mole stronger than carbon-hydrogen bond, and is less susceptible to metabolic oxidation. Fluorine atom significantly changes the hydrophobicity and permeability of such groups as trifluoromethyl and difulromethyl making them highly lipophilic. Moreover, fluorine substitution could increase the binding affinity of the ligand with the targeting protein (21). Due to the described unique properties of fluorine, the introduction of fluorine(s), difluoromethyl, or trifluoromethyl group could modulate the properties of bioactive molecules and lead to substantially improved pharmacological profile (22) (23). Fluorine can also serve as a unique and valuable tool for *in vitro* and *in vivo* ^{19}F NMR studies of protein structures and rug-protein interactions as fluorine is virtually absent in the living tissue (24) (25). Fluorine-containing β-lactams as well as their derivatives are expected to serve as important and useful bioactive compounds for medicinal chemistry and chemical biology.

From previous studies, it is known that the primary sites of metabolism on the paclitaxel molecule for the P450 (CYP) family of enzymes are the para-position of the C-3' phenyl, the meta-position of the C-2 benzoate, the C-6 methylene, and the C-19 methyl groups (26). When a C-H bond is replaced with a C-F bond at these sites, a substantial decrease in the rates of enzymatic oxidation should occur to enhance metabolic stability. The fluorine-containing second-generation taxoids were found to have more than one order of magnitude better activity against drug resistant cell lines as compared with paclitaxel (27). Within the framework of structure-activity relationship studies of fluoro-taxoid anticancer agents the synthesis of difluorovinyl second-generation taxoids was investigated.

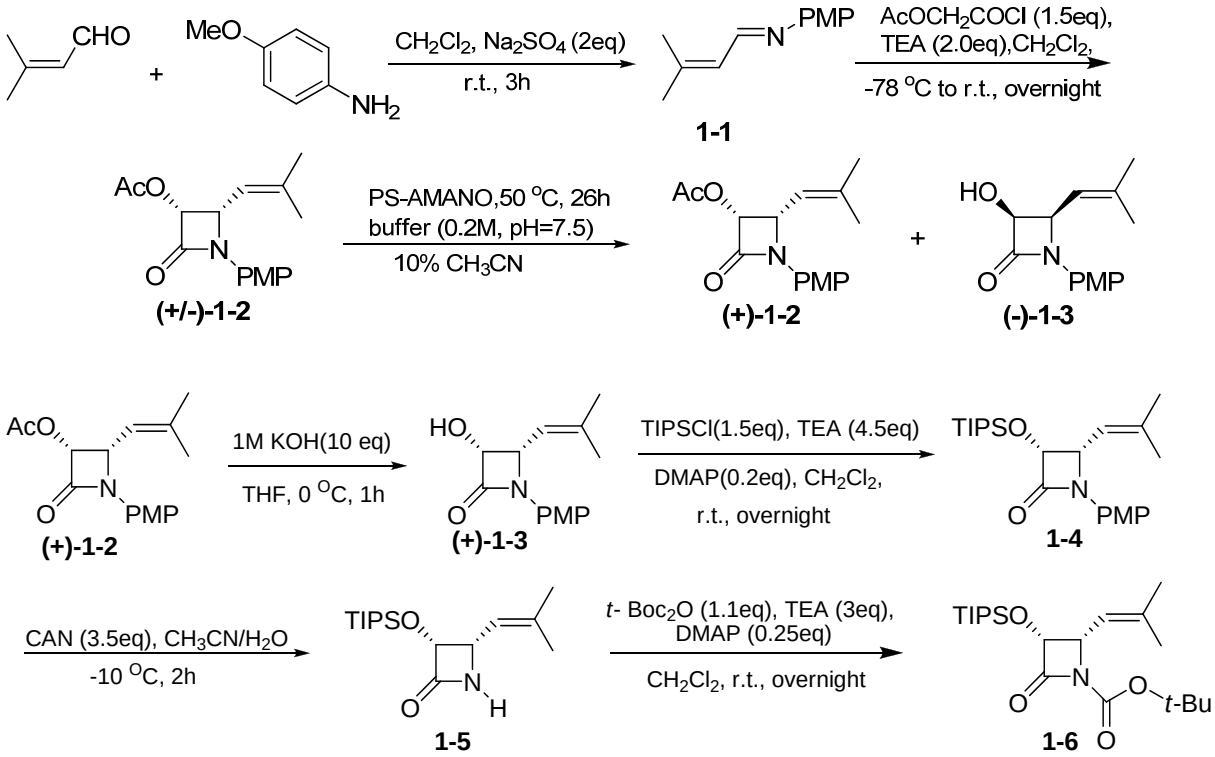
1.2. Results and Discussion

1.2.1 Enzymatic resolution route

One of the methods to obtain the enantiopure compounds is enzymatic resolution. Hydrolytic enzymes were used for enzymatic kinetic resolution. Conventionally, it is an efficient way to generate enantiopure β-lactams from racemic β-lactams. Amano lipase was selected as it is one of the most efficient hydrolytic enzymes that give high enantiomeric purity of the isolated products. With the use of PS-Amano lipase, (+)-β-

lactam, **(+)-1-2 (scheme 1)**, can be obtained with high enantiopurity if the reaction is quenched when the conversion reaches at fifty percent (28) (29).

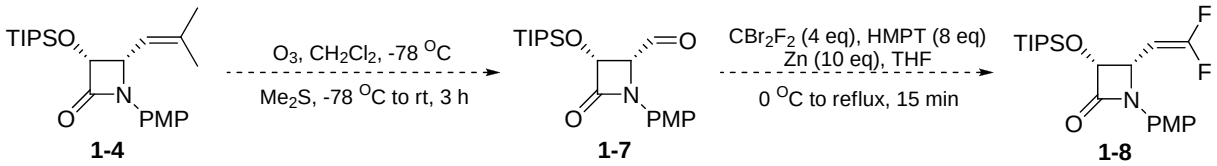
With PS-Amano lipase, the acetate moiety at C-3 of the (*3S,4R*) enantiomer of **1-2** was hydrolyzed. The acetoxy group of the (*3R,4S*) enantiomer of **1-2** was then hydrolyzed and the 3-hydroxyl group was protected with TIPS to give **1-4**. The *p*-methoxyphenyl group was then removed through oxidation with ceric ammonium nitrate , and subsequently, a standard acylation with di-*tert*-butyldicarbonate anhydride gave the desired *β*-lactam, 1-(*tert*-butoxycarbonyl)-3-triisopropylsiloxy-4-(2-methylpropen-2-yl)azetidin-2-one, **1-6**, as shown in s**cheme 1-1**.



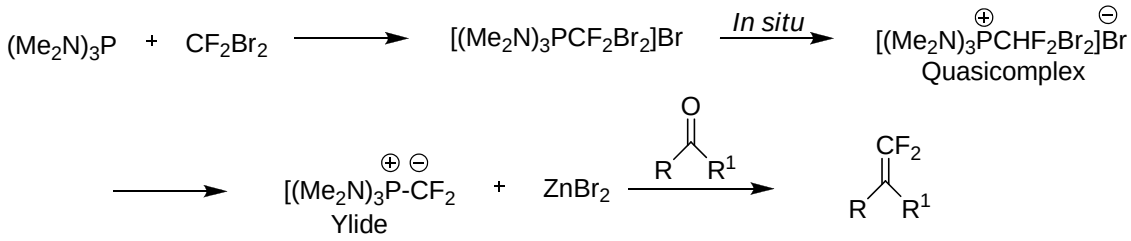
Scheme 1-1: Synthesis of 1-(*tert*-butoxycarbonyl)-3-triisopropylsiloxy-4-(2-methylpropen-2-yl)azetidin-2-one, **1-6**

Besides the synthesis of 4-dimethylvinyl-β-lactam, 4-difluorovinyl-β-lactam can also be synthesized from (*3R,4S*)-3-AcO-β-lactam in a similar manner, and has been carried out by Ojima group members in the past. First, it underwent cycloaddition, enzymatic optical resolution, hydrolysis and TIPS protection, to yield (3*R*,4*S*)-1-PMP-3-TIPSO-4-(2-methyl-2-propenyl)azetidin-2-one (**1-4**). **1-4** was subject to ozonolysis to give (3*R*,4*S*)-1-PMP-3-TIPSO-4-formylazetidin-2-one (**1-7) (Scheme 1-2)**. This was then

transformed to (3*R*,4*S*)-1-PMP-3-TIPSO-4-difluorovinylazetidin-2-one (**1-8)**, using CBr_2F_2, hexamethylphosphoroustriamide (HMPA) and Zn in THF (**Scheme 1-2**). In the difluoromethylation reaction, a highly exothermic reaction occured immediately due to the formation of quasicomplex with zinc and ylide system (**Scheme 1-3**). Finally, the PMP group was removed using CAN followed by carbalkoxylation with Boc_2O to give the desired (3*R,*4*S*)-*N*-Boc-3-TIPSO-4-difluorovinylazetidin-2-one (**2-9)**.



Scheme 1-2: Synthesis of (3*R*,4*S*)-1-PMP-3-TIPSO-4-difluorovinyl-2-one



Scheme 1-3: Mechanism of 1,1-difluoroolefin formation

1.2.2 Synthetic route from a chiral source

In the past, the Ojima group prepared (3*R*,4*S*)-3-AcO-β-lactam through [2+2] ketene-imine cycloaddition followed by the enzymatic optical resolution of racemic β-lactam (27). However, enzymatic resolution method can only give maximum of 50 % yield from the racemic-β-lactam and can be time consuming. An alternative synthesis was proposed with the use of a naturally occurring, less expensive and commercially available starting material- D-mannitol, (2*R,*3*R*,4*R*,5*R*)-hexane-1,2,3,4,5,6-hexaol. This method introduces chiral centers in 4 member ring from the chiral source.

The ketal formation of D-mannitol was performed in acetone in the presence of zinc chloride to give 1,2:5,6-di-*O*-isopropylidene-D-mannitol. This procedure is able to produce the desired product in a relatively larger scale and higher yield using cheap reagents and is beneficial as it can be routinely used in a lab, in large quantities.

In the following step, heterogeneous sodium periodate was employed in dichloromethane that contained diisopropylidene-D-mannitol, followed by the addition of

saturated aqueous sodium carbonate. Sodium carbonate acts as neutralizer. When the reaction was complete, the solvent was removed and dried over sodium sulfate. The oxidant was filtered easily and afford the desired aldehyde **2-2** without further purification. With the obtained aldehyde **2-2**, *p*-anisidine was introduced into the same pot with the addition of magnesium sulfate to form shiff base. Ketene was generated from triethylamine and acetoxyacetyl chloride in the same reaction flask and further reacted with the shiff base to perform [2+2] ketene-imine cycloaddition to yield (2*S*,3*R*)-3-acetoxy-4-[(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]-1-(4-methoxyphenyl)azetidin-2-one (**2-3**).

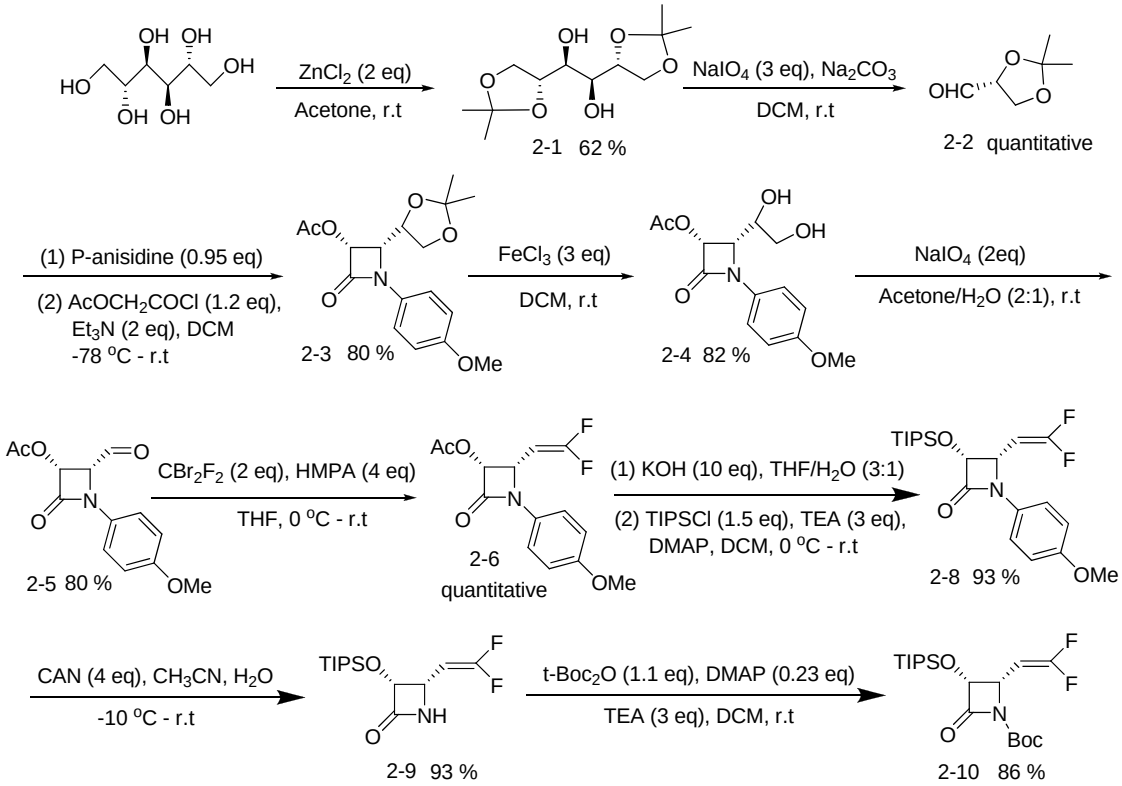
Ketals are widely used as protecting groups in organic synthesis and, as a consequence, many methods have been examined for both their formation and removal. Deprotection of acetonide to furnish diols was accomplished using ferric chloride trihydrate in dichloromethane. The extraction wad done by diluting with ethyl acetate and washing carefully with water and brine. The diol **2-4** was unstable and could change conformation from cis to trans very easily. Therefore, once it is recrystallized, it must be used directly in the following step.

The oxidative cleavage of diol by sodium periodate in acetone-water mixture provided (2*R*,3*R*)-3-acetoxy-4-formyl-1-(4-methoxyphenyl)azetidin-2-one (**2-5)** in good yield. The methodology not only consists of fewer steps but is operationally simple and can be adopted for a large-scale preparation (30).

Enantiopure (2*R*,3*R*)-3-acetoxy-1-(4-methoxyphenyl)-4-formylazetidin-2-one (**2-5)** was converted to (2*S*,3*R*)-3-acetoxy-4-(2,2-difluorovinyl)-1-(4-methoxyphenyl)-4-formylazetidin-2-one (**2-6)** using CBr_2F_2, hexamethylphosphoroustriamide (HMPA) in THF. HMPA was used as the reagent and base. The synthesis for difluorovinyl compounds went through formation of $[(Me_2N)_3P^+\text{-}CF_2Br]Br^-$. The ylide conversion was fast and yielded the desired difluorovinyl moiety within a short period of time. When this difluoronation reaction was repeated, it was found that Zn, which was used to help the formation of quasi-complex, was not necessary. In fact, addition of Zn might have destroyed the desired product to some extent.

In the next step, (3*R*,4*S*)-3-acetoxy-1-PMP-3-TIPSO-4-difluorovinylazetidin-2-one (**2-6)** underwent hydrolysis in THF/H_2O (3:1) with the addition of KOH pellet. The protecting group of the 3-hydroxyl moiety was then changed to triisopropylsilyl (TIPS) in

the present of triethylamine, DMAP and dichloromethane. The removal of the *p*-methoxyphenyl group (PMP) was done by adding cerium ammonium nitrate (CAN) dropwise into the reaction flak that contained water and acetonitrile. Lastly, the desired β-lactam, (3*R*,4*S*)-1-Boc-3-TIPSO-4-difluorovinylazetidin-2-one (**2-9)** was obtained through carbalkoxylation with Boc_2O (**Scheme 1-4**).



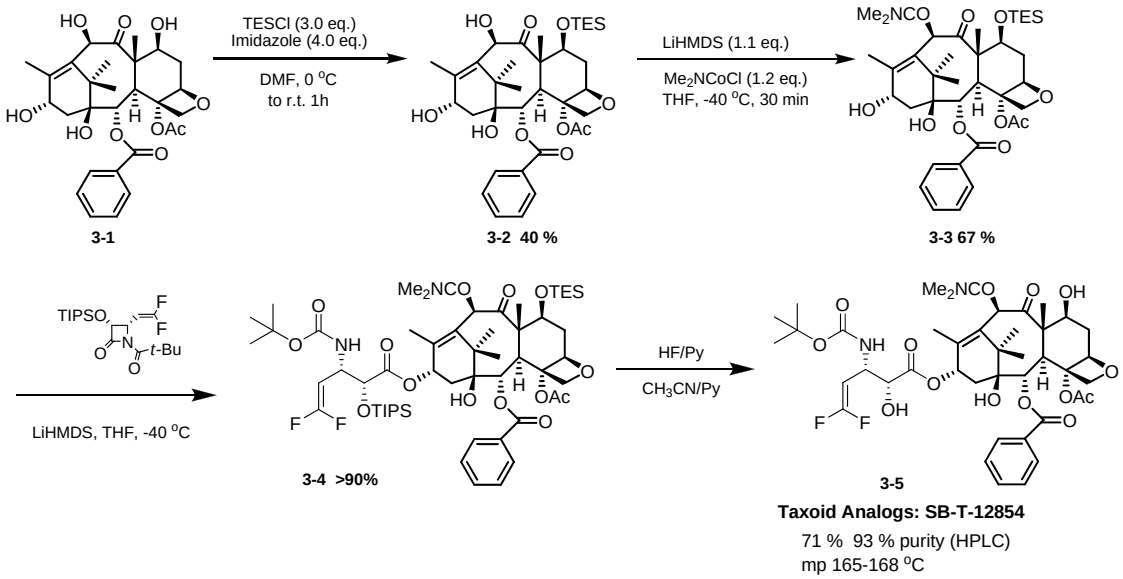
Scheme 1-4: Synthesis of (3*R*, 4*S*)-*N*-Boc-3-TIPSO-4-difluorovinylazetidin-2-one

(3*R*,4*S*)-1-Boc-3-TIPSO-4-difluorovinylazetidin-2-one (**2-10**) was synthesized and used for the synthesis of SB-T-12854.

1.2.3 Synthesis of Taxoid Analog SB-T-12854

With the natural product 10-deacetylbaccatin III (**2-1)**, a selective protection of C7-OH using 3 equivalents of triethylsilyl chloride (TESCl) and an excess of imidazole in dimethylformamide (DMF) solution yielded the protected 7-triethylsilyl-10-deacetylbaccatin III (**2-2)**. The protected baccatin **2-2** was treated with lithium bis(trimethylsilyl)amide (LiHMDS), followed by the addition of *N*-*N*-dimethylcarbamoyl chloride to yield C10-*N*-*N*-dimethylcarbamoyl-7-(triethylsilyl)-10-deacetylbaccatin III

(**2-3)**. The *β*-lactam **1-6** was coupled to baccatin **2-3** to yield C10-*N*-*N*-dimethylcarbamoyl-3'-dephenyl-3'-(2-methyl-2-propenyl)-7-TES-docetaxel (**2-4**) in the presence of LiHMDS. Following global silyl group deprotection using HF-pyridine conditions gave taxoid **SB-T-12854** as depicted in **Scheme 1-3**. The melting point of SB-T-12854 was 165-168 ˚C (Literature value: 166-170 ^{o}C) (31), and the optical rotation value was $[\alpha]_D^{22}$ – 82.2 (c 1.18, DCM); the specific optical rotation were not measured in the past synthesis and were done now to stress the importance of chirality.



Scheme 1-5: Synthesis of Taxoid Analogs, **SB-T-12854**

General Method and Materials:

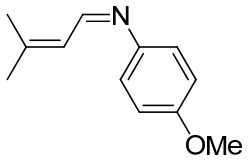
NMR spectra were measured on a Bruker AC-25- NMR spectrometer or a Varian 300 NMR spectrometer using tetramethylsilane as the internal standard. Melting point was measured on a Thomas Hoover Capillary melting point apparatus. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter. TLC was performed on Merck DC-alufolien with Kieselgel 60F-254 and column chromatography was carried out on silica gel 60(Merck; 230-400 mesh ASTM). Chiral HPLC analysis for the determination of enantiomeric excess was carried out with a Waters HPLC assembly. HPLC assembly comprises Waters M45 solvent delivery system, Waters Model 680 gradient controller, Water M440 detector (at 254 nm) equipped with a Spectra Physics Model SP4270 integrator. The system uses a DAICEL-CHIRACEL OD chiral column (25 x 0.46 cm i.d.), employing hexane/2-propanol (99.5/0.5, v/v) as the solvent system with a flow rate

of 1.0 ml/min. HPLC analysis for determination of isomeric ratio was carried out with the same Water HPLC assembly using 5u Spherical Silica column employing hexane/2-propanol/dichloromethane (15/1/1, v/v/v) as the solvent system with a flow rate 1.0 ml/min, or hexane/2-propanol/dichloromethane (10/1/1, v/v/v) as the solvent system with a flow rate 1.4 ml/min. Elemental analysis were performed at M-H-W Laboratory, Phoenix, AZ. High resolution mass spectra were obtained from the California, Riverside Mass Spectrometry Facility, Riverside, CA; or Mass Spectrometry Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL.

The chemicals were purchased from Aldrich and Sigma and purified before use by standard methods. Dichloromethane was distilled under nitrogen from calcium hydride immediately before use.

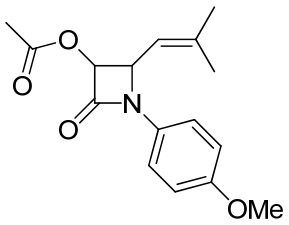
1.3. Experimental Section

***N*-(4-Methoxyphenyl)-3-methyl-2-butenaldimine (1-1):** (32)



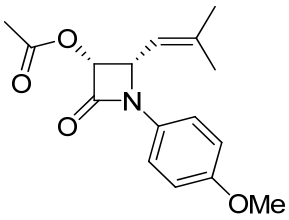
To a mixture of recrystallized *p*-anisidine (1.004 g, 8.152 mmol) and anhydrous $NaSO_4$ in 25 mL CH_2Cl_2 was added 3-methyl-2-butenal (0.966 mL) dropwise. The mixture was stirred at room temperature for 3 h under dark conditions. The solution was vacuum filtered and evaporated to yield crude **1-1** as yellow viscous oil, which was immediately used for the next reaction without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ1.95 (s, 3 H), 2.01 (s, 3 H), 3.80 (s, 3H), 6.20 (d, J = 9.5 Hz, 1 H), 6.89 (d, J = 7.0 Hz, 2H), 7.11 (d, J= 7.0 Hz, 2H), 8.38 (d, J = 9.5 Hz, 1 H). All data are in agreement with literature values.

(±)-1-(4-Methoxyphenyl)-3-acetoxyl-4-(2-methylprop-1-enyl)azetidin-2-one ((+/-)-1-2): (32)



To the resulting crude **1-1** in CH_2Cl_2 (40 mL) was added triethylamine (2.27 mL), followed by dropping acetoxyacetyl chloride (1.36 mL) at -78 ˚C. The reaction mixture was stirred for 1 h, and then slowly allowed to warm up to room temperature before quenching with CH_2Cl_2. The organic layer was washed three times with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. Column chromatography of the residue on silica gel (hexane:ethyl acetate = 3:1) yielded the desired racemic β-lactam **1-2** (1.718g, 73 % after two steps) as a white solid: mp 105-108 ^oC (Literature value: mp 107-109 °C;); ^1H NMR (300 MHz, $CDCl_3$) δ1.79 (s, 3 H, H on CH_3), 1.82 (s, 3H, H on CH_3), 2.12 (s, 3 H, H on CH_3 of acetate), 3.79 (s, 3 H, H on CH_3 of PMP), 4.97 (dd, J = 9.6 Hz, 4.8 Hz, 1 H, H on CH of isobutenyl), 5.14 (dm, J = 9.6 Hz, 1 H, H on C4), 5.81 (d, J = 5.7 Hz, 1 H, H on C3), 6.87 (d, J = 6.9, 2 H, H on benzene ring), 7.20 (d, J = 7.2 Hz, 2 H, H on benzene ring). All data are in agreement with literature values.

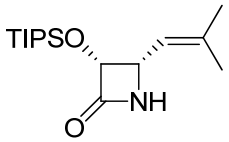
Enantioselective hydrolysis of β-lactam ((+)-1-2): (33)



The obtained **1-2 (**1.718 g, 5.938 mmol) was suspended in 200 mL of 0.2 M sodium phosphate buffer (pH = 7.5) and 16 mL of acetonitrile was added 301.2 mg of the PS Amano lipase, and the mixture was vigorously stirred at 50 °C. After 25 h, the ^1H NMR showed the conversion of the reaction was 50 %. The reaction mixture was filtered through celite and extracted with CH_2Cl_2. The combined organic layers was washed with brine and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by

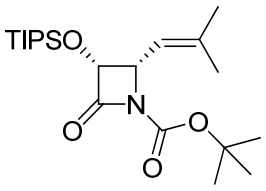
column chromatography on silica gel (hexane:ethyl acetate = 4:1) to yield (+)-*cis*-l-(4-methoxyhenyl)-3-acetoxy-4-(2-methylprop- 1-enyl)-2-one (**1-2)** 26 %, as white solid, and (-)-*cis*-l-(4-methoxyhenyl)- 3-hydroxy-4-(2-methylprop-1-enyl)- 2-one (**1-3**) 12%: ^{1}H NMR (300 MHz, $CDCl_3$) δ1.79 (s, 3 H, H on CH_3), 1.82 (s, 3H, H on CH_3), 2.1 (s, 3 H, H on CH_3 of acetate), 3.78 (s, 3 H, H on CH_3 of PMP), 4.97 (dd, *J* = 9.6 Hz, 4.5 Hz, 1 H, H on CH of isobutenyl), 5.14 (dm, *J* = 9.6 Hz, 1 H, H on C4), 5.81 (d, *J* = 4.8 Hz, 1 H, H on C3), 6.86 (d, *J* = 6.9, 2 H, H on benzene ring), 7.31 (d, *J* = 7.8 Hz, 2 H, H on benzene ring). All data are in agreement with literature values.

3-Triisopropylsiloxy-4-(2-methylpropen-2-yl)azetidin-2-one (1-5): (32)



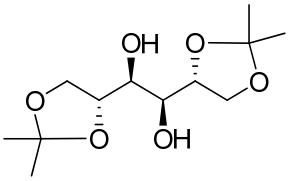
To a solution of *N*-PMP-β-lactam (**1-4)**, (502 mg, 1.24 mmol) in 41 mL of acetonitrile and 41 mL of water, was added dropwise a solution of cerium ammonium nitrate (2.72 g, 4.96 mmol) in 6 mL of water at – 10 ^{o}C. The reaction mixture was stirred for 3 h before quenched with saturated Na_2SO_3 solution. The aqueous layer was extracted with ethyl acetate three times and the combined organic layer was washed with water, dried over $MgSO_4$ and concentrated. The residue was purified on a silica gel column using hexane:ethyl acetate (4:1) as the eluent to afford **1-5** in 47 % yield (173 mg) as a white solid; mp 84-86 ^{o}C (Literature value: 85-86 ^{o}C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97-1.21 (m, 21 H), 1.68 (d, J = 2.3 Hz, 3H), 1.19 (d, J = 2.3 Hz, 3 H), 4..43 (dd, J = 9.5, 4.7 Hz, 1 H), 4.98 (dd, J = 4.7, 2.3 Hz, 1H), 5.31 (d, J = 9.5 Hz, 1 H). All data are in agreement with literature values.

1-(*tert*-Butoxycarbonyl)-3-triisopropylsiloxy-4-(2-methylpropen-2-yl)azetidin-2-one (1-6): (32)



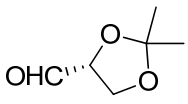
To a solution of N-H-β-lactam (173 mg, 0.582 mmol) and DMAP (17.8 mg, 0.15 mmol) in 30 mL CH_2Cl_2, was added dropwise triethylamine (12 mL) and a solution of di-*tert*-butyldicarbonate (140 mg, 0.640 mmol) in 2 mL of CH_2Cl_2. The mixture was stirred over night at room temperature before quenched with saturated NH_4Cl and extracted with ethyl acetate. The combine extracts were dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was purified on a silica gel column (hexane:ethyl acetate = 4:1) to afford **1-6** as a colorless oil in 91 % yield: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.02-1.2 (m, 21 H), 1.48 (s, 9 H), 1.77 (d, J = 1.0 Hz, 3 H), 1.79 (d, J = 1.0 Hz, 3H), 4.75 (dd, J = 9.8, 5.6 Hz, 1 H), 4.98 (d, J = 5.6 Hz, 1H), 5.28 (dd, J = 9.8, 1.0 Hz, 1H). All data are in agreement with literature values.

1,2:5,6-Di-O-isopropylidene-D-mannitol (2-1): (34)



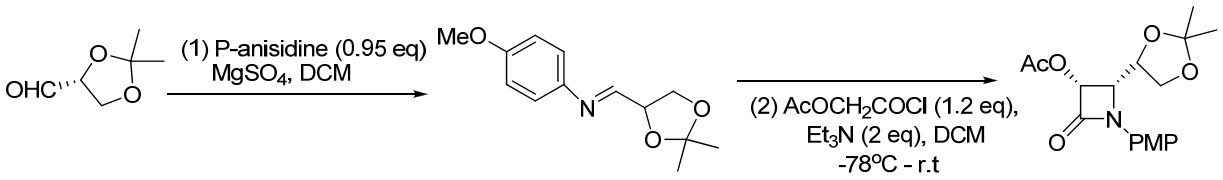
To a suspension of D-mannitol (7.0 g, 38.4 mmol) in dry acetone (100 mL) was added dropwise a solution of zinc chloride in diethyl ether (2.0 M, 76.8 mmol) in ice bath. The mixture was stirred for 30 min at 0 ^{o}C, and 24 h at room temperature. To the reaction mixture was added ethyl acetate (100 mL), saturated $NaHCO_3$ (50 mL) and water (50 mL). The precipitate was filtered, and washed with ethyl acetate (2x50 mL). The filtrate was separated. The organic layer was washed with water (100mL), brine (30 mL), dried over anhydrous Na_2SO_4 and concentrated by rotavapor. The crude product was recrystallized from hexane (200 mL) to afford product **2-1** as white crystals (6.3 g, 62 %): ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 6 H), 1.42 (s, 6 H), 2.70 (d, J = 6.7, 2H,), 3.75 (m, 2 H), 3.98 (m, 2 H), 4.10–4.22 (m, 4 H). All data are in agreement with literature values.

(*R*)-2,3-*O*-Isopropylideneglyceraldehyde (2-2): (34)



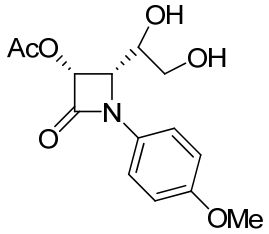
To a solution of 1,2:5,6-diisopropylidene-D-mannitol (2.0 g, 7.6 mmol) in dichloromethane (50 mL) was added $NaIO_4$ (3.25 g, 15.2 mmol) followed by saturated aqueous Na_2CO_3 (3 mL). The mixture was stirred for 5 h at room temperature. The reaction mixture was dried over $MgSO_4$, and filtered. The solvent was removed by rotavapor to give a fairly pure product **2-2** as a colorless oil that did not need to be purified for further reaction (1.9 g, 98 %): ^{1}H NMR (250 MHz, CDCl3) δ 1.40 (s, 3 H), 1.45 (s, 3 H), 4.08 (m, *J* = 4.85, 1 H, 3 H), 4.16 (m, 1 H, 3 H), 4.37 (m, 1 H, 2 H), 9.70 (d, *J* = 1.9 Hz, 1 H). All data are in agreement with literature values.

1-PMP-(3*R*)-acetoxy-(4*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)-azetidin-2-one (2-3) (36)



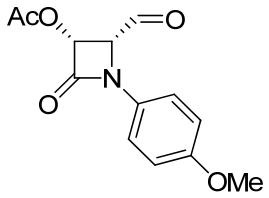
To a stirred solution of aldehyde **2-2** (1.56 g, 12.0 mmol) in CH_2Cl_2 (60 mL) was added *p*-anisidine (1.4 g, 11.5 mmol) followed by $MgSO_4$ (2 g) at room temperature. The mixture was stirred for 1 h, and filtered. To the yielded Schiff base **3** in the filtrate was added triethylamine (2.5 mL, 23 mmol). The mixture was cooled down to -78 ^{o}C and added dropwise a solution of acetoxyacetyl chloride (2.26 g, 13.8 mmol) in dry CH_2Cl_2 (100 mL) with stirring under a nitrogen atmosphere. The reaction mixture was slowly warmed to room temperature, and washed with water and brine. The organic layer was dried over Na_2SO_4, and evaporated to about 30 mL. To the crude product residue was added hexane until it became lightly cloudy, a pale brown needle crystals of the product **2-3** was obtained (3.28 g, 85 %): mp 159-160 ^{o}C (literature value: 163 ^{o}C); $[\alpha]_D^{23}$ + 89.5 (c 1.05, DCM) (literature value: $[\alpha]_D^{26}$ + 101.3 (c 0.5, MeOH)); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75-6.90 (dd, AB pattern, 4H), 6.05 (d, J = 5.7 Hz, 1 H), 4.45 (m, 2 H), 4.05 (m, 1 H), 3.8 (s, 3 H), 3.65 (m, 1 H), 2.15(s, 3 H), 1.50 (s, 3 H), 1.30 (s, 3 H); ESI MS m/z 336.1 $[M+H]^+$. All spectra are in agreement with literature values (34). Enantiopurity was determined by HPLC with a chiral column (OD-H column, eluent IPA/Hex=10/90, flow rate: 1 mL/min, t_R= 19.5min).

1-PMP-(3*R*)-acetoxy-(4*S*)-(1,2-dihydroxy-ethyl)azetidin-2-one (2-4): (36)



To a solution of β-lactam **2-3** (1.61 g, 4.81 mmol) in dichloromethane (40 mL) was added$FeCl_3$ ·trihydrate (9.2 g, 17.6 mmol) at room temperature and stirred for 3 h. After the completion of the reaction monitored by TLC, the mixture was washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated by rotavapor. The crude product was recrystallized in a mixture of ethyl acetate and hexane, affording a white solid **2-4**. Or, without purification the crude product could be used for further reaction (1.16 g, 82 %): mp 131-132 ^oC (no literature value); $[\alpha]_D^{23}$ + 97.1 (c 1.02, DCM) (no literature value); ^1H NMR (300 MHz, $CDCl_3$) δ 7.46 (m, 2H), 6.87 (m, 2 H), 6.03 (d, J = 5.4 Hz, 1 H), 4.50 (t, J = 5.4 Hz, 1H), 4.10 (q, J = 5.1 Hz, 1 H), 3.79 (s, 3 H), 3.63 (d, J = 6.0 Hz, 2 H), 2.20 (s, 3 H); ESI MS m/z 296.1 $[M + H]^+$. All spectra are in agreement with literature values.

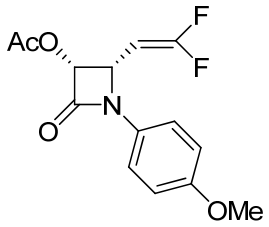
1-PMP-(3*R*)-acetoxy-(4*S*)-formyl-azetidin-2-one (2-5): (36)



To a solution of diol **2-4** (1.83 g, 6.20 mmol) in acetone-water (2:1 v/v, 15 mL) was added powdered $NaIO_4$. The solution was stirred for 2 h at room temperature. After completion of the reaction, monitored by TLC, the reaction mixture was diluted with ethyl acetate (50 mL), washed with water and brine, dried over Na_2SO_4 and concentrated under reduced pressure to give the crude aldehyde. Purification by flash chromatography on silica gel (hexane-EtOAc gradient) afforded product **2-5** as white crystals (1.30 g, 80 %). Or, the crude product was used for further reaction directly without purification: mp 136-137 ^oC (literature value: 135 ^oC); $[\alpha]_D^{23}$ + 134.9 (c 1.09, DCM) (no literature value); ^1H NMR (300 MHz, $CDCl_3$): δ 9.71 (d, J = 3.0 Hz, 1 H), 7.24 (m, 2 H), 6.85 (m, 2 H),

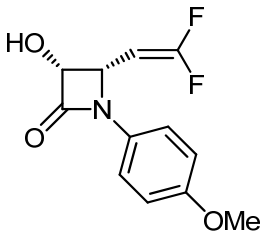
5.98 (d, J = 5.1 Hz, 1 H), 4.72 (dd, J = 3.0, 2.4 Hz, 1 H), 3.77 (s, 3 H), 2.12 (s, 3H); ESI MS m/z 264.13 $[M + H]^+$. All spectra are in agreement with literature values.

1-PMP-(3*R*)-acetoxy-(4*S*)-difluorovinylazetidin-2-one (2-6): (37)



To mixture of aldehyde **2-5** (2.0 g, 7.6 mmol) and dibromodifluoromethane (4.78 g, 22.8 mmol) in dry THF (200 mL) was added hexamethylphosphorous triamide (HMPA) (7.44 g, 45.6 mmol) under N_2 at 0 ^oC. After addition, the mixture was allowed to warm to room temperature ad stirred for 1 h. The mixture was diluted with ethyl acetate (250 mL), washed with water and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give yellow oil. The crude residue was purified by flash chromatography on silica gel to afford **7** as colorless crystals (2.1 g, 92%): mp 119-120 ^oC (no literature value); $[\alpha]_D^{23}$ -13.7 (c 0.51, DCM) (no literature value); ^1H NMR (300 MHz, $CDCl_3$) δ 7.32 (d, J = 9.0 Hz, 2H), 6.87 (d, J = 9.0 Hz, 2H), 5.93 (d, J = 5.1 Hz, 1H), 5.01 (dd, 1H), 4.39 (dd, J = 10.5, 16.5 Hz, 1H), 3.79 (3 H, s), 2.20 (s, 3H); ESI MS m/z 297.8 $[M + H]^+$ All spectra are in agreement with literature values. Enantiopurity was determined by HPLC with a chiral column (OD-H column, eluent IPA/Hex=10/90, flow rate: 1 mL/min, t_R= 13.6 min).

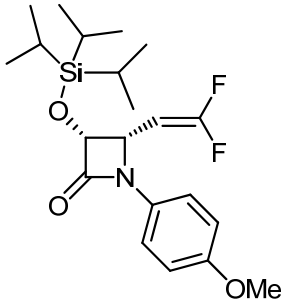
1-PMP-(3*R*)-hydroxyl-(4*S*)-difluorovinylazetidin-2-one (2-7) (32)



To a solution of β-lactam **2-5** (1.2 g, 4.0 mmol) in THF-H_2O (20 mL, 3:1, v/v) was added KOH pellet (2.2 g, 40 mmol) at 0 ^oC. The mixture was stirred for 20 min, and allowed to warm to room temperature with stirring for 20 min. The reactioin mixture was

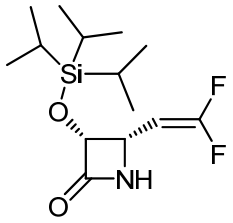
diluted with ethyl acetate (100 mL), washed with water and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give a crude product as oil. The residue was purified by flash chromatography by silica gel to afford **8** as colorless crystals, or directly used for the next step (2.1 g, 92%): mp 117-118 ^oC (no literature value); $[\alpha]_D^{23}$ +68.9 (c 0.45, DCM) (no literature value); ^1H NMR (300 MHz, $CDCl_3$) δ 7.29 (d, J = 9.0 Hz, 2H), 6.85 (d, J = 9.0 Hz, 2H), 5.14 (d, J = 3.0 Hz, 1H), 4.89 (d, J = 6.0 Hz, 1H), 4.68 (dd, J = 12.0, 12.0 Hz, 1H), 4.15 (s, 1 H), 3.78 (s, 3H); ESI MS m/z 256.0 $[M + H]^+$. Enantiopurity was determined by HPLC with a chiral column (OD-H column, eluent IPA/Hex=10/90, flow rate: 1 mL/min, t_R= 11.5 min).

1-PMP-(3*R*)-(TIPS-O)-(4*S*)-difluorovinylazetidin-2-one (2-8): (37)



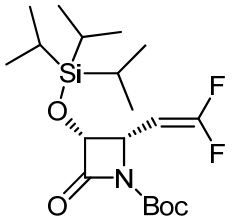
To a mixture of β-lactam **2-6** (800 mg, 2.7 mmol) in dry dichloromethane (DCM) (50 mL), triethylamine (920 mg, 8.1 mmol) and 4-*N*,*N*-dimethylaminopyridine (DMAP) (0.6 mg) was added dropwies triisopropylsilyl chloride (TIPS-Cl) (780 mg, 4.0 mmol) under N_2 at 0 ^oC. The mixture was stirred for 30 min at 0 ^oC, and then allowed to warm to room temperature for overnight. The reaction mixture was diluted with additional DCM (50 mL), washed with water and brine, and dried over Na_2SO_4. The filtrate was concentrated under reduced pressure to give crude product **2-8** as light yellow oil. Crude residue was directly used for the next step without further purification (1.12 g, 93%): ^1H NMR (300 MHz, $CDCl_3$) δ 7.32 (d, J = 9.0 Hz, 2H), 6.87 (d, J = 9.0 Hz, 2H), 5.14 (d, J = 5.1 Hz, 1H), 4.83 (m, 1H), 4.54 (ddd, J = 1.5, 6.3, 16.5 Hz, 1H), 3.79 (3 H, s), 1.08-1.15 (m 21H); ESI MS m/z 412.2 $[M + H]^+$. All spectra are in agreement with literature values.

(3*R*)-Triisopropylsiloxy-(4*S*)-difluorovinylazetidin-2-one (2-9): (37)



To a solution of *N*-PMP-β-lactam **2-8**(688 mg, 1.6 mmol) in acetonitrile (50 mL) was added drolpwise a solution of ceric ammonium nitrate (CAN) (3.74 g, 6.7 mmol) in water (50 mL) at -10 ^{o}C. The mixture was stirred for 20 min, and allowed to warm to room temperature with stirring for 2 hours. The reaction mixture was quenched with water and saturated Na_2SO_3, and extracted with ethyl acetate (3 x 50 mL). The combined organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated. The crude product was purified though flash chromatography on silica gel to afford **2-9** as yellowish oil (469 mg, 92%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.59 (bs, 1H), 5.04 (dd, J = 1.6, 2.4 Hz, 1H), 4.44-4.54 (m, 2H), 1.03-1.18 (m, 21H); ESI MS m/z 306.1 $[M + H]^+$. All spectra are in agreement with literature values.

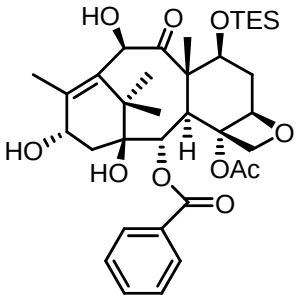
1-*t*-Boc-(3*R*)-(TIPS-O)-(4*S*)-difluorovinylazetidin-2-one (2-10): (37)



To a solution of difluorovinyl-β-lactam **2-9** (515 mg, 1.69 mmol), triethylamine (0.82 mL, 5.06 mmol) and DMAP (48 mg, 0.38 mmol) in DCM (10 mL), was added Boc_2O (406 mg, 1.85 mmol) at room temperature. The mixture was stirred for 18 hrs and quenched with water. The reaction mixture was diluted with additional DCM and organic layer was washed with water and brine, dried over Na_2SO_4 and concentrated. The crude residue was purified through flash chromatography on silica gel to afford final product **2-10** as light yellow oil (586 mg, 86%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.05 (d, J = 5.7 Hz, 1H), 4.75 (dddd, J = 0.9, 2.4, 5.1, 9.0, 1H), 4.47 (ddd, J = 1.6, 13.8, 23.7 Hz), 1.49 (s, 9H), 1.03-

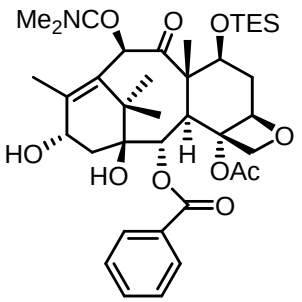
1.18 (m, 21H); ESI MS m/z 406.3 $[M + H]^+$. All spectra are in agreement with literature values.

7-Triethylsilyl-10-deacetylbaccatin III (3-2): (13)



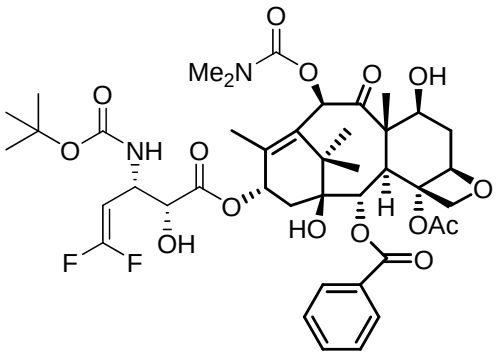
To a solution of 10-deacetylbaccatin III (**3-1)** (2.04 g, 3.75 mmol) and imidazole (1.07 g, 15.76 mmol) in *N*,*N*-dimethylformamide (DMF, 90 mL) was added chlorotriethylsilane (1.70 mL, 11.25 mmol) dropwise *via* syringe at 0 ^oC, and then the reaction mixture was stirred for 2 h at room temperature and diluted with ethyl acetate. The mixture was then washed with water, brine, drived over anhydrous Na_2SO_4 and concentrated. The crude product was purified on a silica gel column using hexanes:EtOAc (1:1) as eluent to give **3-2** as a white solid (990 mg, 40 %): ^1H NMR (300 MHz, $CDCl_3$) δ 0.56 (m, 6H), 0.94 (m, 9 H), 1.08 (s, 6H), 1.59 (d, J = 2.5 Hz, 1H), 1.73 (s, 3H), 1.90 (dt, 1 H), 2.05 (d, J = 4.8 Hz, 1H), 2.08 (s, 3H), 2.24 (s, 1H), 2.28 (s. 3H), 2.48 (ddd, 1H), 3.95 (d, J = 7.1Hz) 4.16 (d, J = 8.3 Hz, 1H), 4.25 (s, 1H), 4.31 (d, J = 8.3 Hz, 1H), 4.40 (dd, J = 6.4, 10.5 Hz, 1H), 4.85 (t, 1H), 4.95 (d, J = 8.0 Hz, 1H), 5.17 (s, 1H), 5.60 (d, J = 7.0 Hz, 1H), 7.47 (t, J = 7.5 Hz, 2H), 7.60 (t, J = 7.5 Hz, 1H), 8.10 (d, J = 7.3 Hz, 2H); ESI MS m/z 659.8 $[M + H]^+$. All data are in agreement with the literature values.

7-Triethylsilyl-10-deacetyl-10-dimethylcarbamoylbaccatin III (3-3): (13)



To a solution of 706 mg (1.07 mmol) of 7-triethylsilyl-10-deacetylbaccatin III (**3-2**) in 10 mL of THF was added 1.5 mL of LiHMDS at -40 °C. After the reaction mixture was stirred for 5 min, 0.14 mL of dimethylcarbamoyl chloride was added dropwise. The mixture was then allowed to stir for 30 min at -40 °C. The solution was then quenched with saturated aqueous NH_4Cl solution, and extracted three times with ethyl acetate. The combined organic layers were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification was done by column chromatography on silica gel using hexanes:EtOAc (3:1) as eluent to give **3-3** as a white solid (521 mg, 67 %): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.57 (m, 6H), 0.91 (m, 9H), 1.21 (s, 3H), 1.27 (s, 3H), 1.69 (s, 3H), 1.84 (dt, 1H), 2.21 (s, 2H), 2.26 (s, 3H), 2.29 (s, 2H), 2.49 (m, 1H), 2.95 (s, 3H), 3.09 (S, 3H), 3.91 (d, J = 6.9 Hz, 1H), 4.12 (d, J = 8.4 Hz, 1H), 4.30 (d, J = 8.4 Hz, 1H), 4.48 (dd, J = 6.7 Hz, 10.2 Hz, 1H), 4.84 (t, 1H), 4.97 (d, J = 9.0 Hz, 1H), 5.64 (d, J = 6.9 Hz, 1H), 6.40 (s, 1H), 7.46 (t, 2H), 7.59 (t, 1H), 8.11 (d, 2H); ESI MS m/z 730.8 $[M + H]^+$. All spectra are in agreement with the literature values.

3-Dephenyl-3-(2,2-difluorovinyl)-10-dimethylcarbamoyldocetaxel (3-5, SB-T-12854): (13)



To a solution of **3-3** (511 mg, 0.700 mmol) and β-lactam **2-9** (426 mg, 1.05 mmol) in 8 mL of THF was added LiHMDS (1.05 mL, 1.05 mmol) at -40 °C. The solution was stirred for 1 h at -40 °C before quenched with saturated aqueous NH_4Cl solution and extracted with ethyl acetate. The organic layers were combined and dried over anhydrous Na_2SO_4 and filtered. The solvent was removed under reduced pressure and the residue was purified on a silica gel column using hexanes:EtOAc (8:1) as the eluent to give **3-4** as a white solid (705 mg, 82 %).

To a solution of **3-4** in CH_3CN and pyridine was added HF-pyridine at 0 ^oC and the reaction mixture was warmed to room temperature and then stirred overnight. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ solution and diluted with ethyl acetate. The organic layer was washed with saturated aqueous $CuSO_4$ solution, water and brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was removed and residue was purified on a silica gel column using hexanes: EtOAc (1:1) as the eluent to afford **SB-T-12854** in 71 % yield, as a white solid: mp 165-168 ^oC (Literature value: 166-170 ^oC); $[\alpha]_D^{22}$ – 82.2 (c 1.18, DCM): ^1H NMR (400 MHz, $CDCl_3$) δ 1.15 (s, 3H), 1.25 (m, 3H), 1.30 (s, 9H), 1.67 (s, 3H), 1.84 (m, 1H), 1.89 (m, 4H), 2.31 (m, 2H), 2.38 (s, 3H), 2.53 (ddd, J=6.8, 9.6, 15.2 Hz, 1H), 3.64 (d, J=5.6 Hz, 1H), 3.80 (d, J=6.8 Hz, 1H), 4.17 (d, J=8.4 Hz, 1H), 4.29 (m, 2H), 4.44 (m, 1H), 4.57 (dd, J=10.0, 25.2 Hz, 1H), 4.86 (t, J=8.8 Hz, 1H), 4.97 (d, J=9.2 Hz, 1H), 5.02 (d, J=9.6 Hz, 1H), 5.66 (d, J=7.2 Hz, 1H), 6.24 (m, 2H), 7.49 (t, J=7.6 Hz, 2H), 7.59 (t, J=7.2 Hz, 1H), 8.10 (d, J=7.6 Hz, 2H) (13); ESI MS m/z 865.8 $[M + H]^+$. All data are in agreement with the literature values.

1.4. References

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Chapter II

Synthetic Studies on Benzimidazole-Based Antituberculosis Agents

2.1. Introduction

2.1.1 Therapeutic agent targeting FtsZ

Tuberculosis, or Tubercle Bacillu (TB), is an infectious bacterial disease caused by bacterium *Mycobacterium tuberculosis*. It most commonly affects the lungs, but has the potential to attack the central nervous system, circulatory system, lymphatic system, bones and joints. TB spreads through the respiratory system and can form epidemic easily. The disease exists in two forms, the active form and the latent form. In both cases, patients will have a positive respond to their tuberculin skin test. In active TB, symptoms are present and the infection is contagious. On the contrary, latent tuberculosis means that the organisms have infected the person but does not show any symptoms. This is because the immune system is able to control the infection in the primary stage and prevent the disease from spreading. However, it is possible for the latent form disease to develop into full-blown TB later in life if they are not treated adequately (38).

Recent statistics from WHO estimate that there are about 8.8 million new TB cases every year with a global mortality rate of 24% (39). Particularly, when TB meets HIV, the combination is lethal as each speeding the other's progress. Someone who is HIV-positive is more likely to be infected with TB compared to those who are HIV-negative. TB is a leading cause of death among people who are HIV-positive. Although treatments of TB are available, there are documented surveys that indicate TB resistant strains in every country. In some cases, the strains are resistant to a single drug, but in other, the strains maybe resistant to all major anti-TB drugs. Treating drug-resistant TB is much more challenging and expensive as it takes up to two years costing US $250,000 per patient (40). The cause of drug-resistant TB is due to poor and inadequate local-control chemotherapeutics (41). It may also be caused by inconsistent or partial treatment, if patients do not take all their medicines regularly for the required period of time.

A particularly dangerous form of drug-resistant TB is multidrug-resistant TB (MDR-TB), which is defined as the disease caused by TB bacilli resistant to at least isoniazid and rifampicin, the two most powerful anti-TB drugs. The emergence of extensively drug-resistant (XDR) TB, particularly in settings where many TB patients are also infected with HIV, poses a serious threat to TB control, and confirms the urgent need to strengthen basic TB control and management of drug-resistant TB. As mentioned, the

emergence of multi-drug resistant *Mycobacterium tuberculosis* (Mtb) strains has also made many of the current available anti-TB drugs ineffective. Under the pressing need to identify new drug targets, a potential anti-TB agent targeting to Mtb FtsZ was discovered in the past few years (42).

FtsZ, filamental temperature-sensitive protein Z, is a prokaryotic homologue of the eukaryotic protein tubulin, and is the most abundant cell division protein (43). Like tubulins that polymerize to form microtubules, FtsZ can polymerize and form a highly dynamic cytokinetic ring called the Z ring in a GTP-dependent manner (44). From the experiment measuring FtsZ-GFP fluorescence recovery after photobleaching (FRAP), it has been shown that Z ring is extremely dynamic and is able to continuously remodel itself with a rate comparable to that of microtubules (45). The highly dynamic nature of the Z ring is due to FtsZ's capacity of GTP-dependent, reversible polymerization. The constant balance between assembly and disassembly means that small changes in FtsZ polymerization dynamics are able to trigger large and rapid changes in FtsZ's structure in the cell (46).

FtsZ is the first protein to move to the division site and is essential for recruiting other proteins that produce a new cell wall between the dividing cells at the septum site (46). Assembly of FtsZ is regulated by FtsZ interacting proteins, which include stabilizing factors, such as ZapA, ZipA and FtsA, as well as destabilizing factors, such as SulA, EzrA and MinCD (48). The amount of FtsZ in *E. coli* has been estimated to be about 15,000 molecules/cell (49), but only one third of the cellular FtsZ pool is present in the ring at a given time (50).

FtsZ is widely conserved and is present in virtually all eubacteria as well as in many eukaryotic organelles. It is at the top of the hierarchy of assembly of division proteins, which means that in general, all other division proteins require FtsZ for targeting to the division site. Certain *ftsZ* mutations that alter the form of the Z ring in the cell can also affect the gross morphology of cell division (51). Giving that the inactivation of FtsZ or alteration of its assembly leads to inhibition of Z ring and septum formation, Z ring plays key role in construction of the cell as well as in coordination of the process of bacterial cell division. Accordingly, FtsZ is a very promising target for new antimicrobial drug development.

2.1.2 Mechanism of inhibition of tubulin polymerization

Two examples of inhibitors of tubulin polymerization are albendazole and thiabendazole (**Figure 2-1**). Slayden *et al*. (52) found that these derivatives of benzimidazole interfere and delay *Mycobacterium tuberculosis* cellular division processes inhibitory activity against Mtb (MIC_{99} 16 µg/mL).

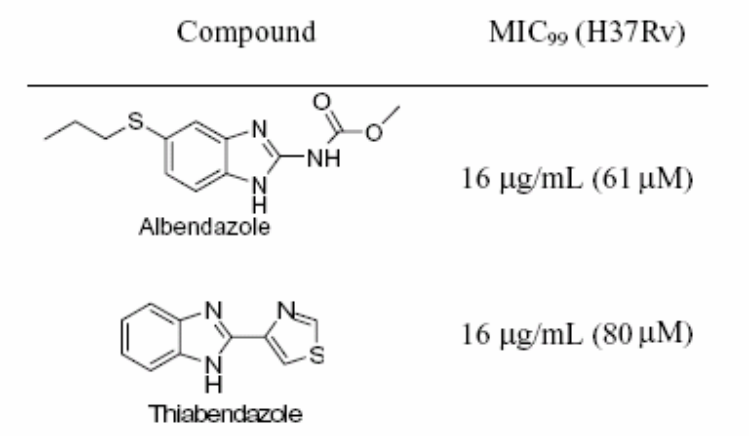


Figure 2-1: Albendazole, thiabendazole - MIC_{99} values against Mtb cells

Further investigation of novel drug that inhibits FtsZ polymerization had been undertaken by examining the structure-activity relationship (SAR) studies of these two pharmacophores. Based on the structural similarity, it was proposed that the benzimidazole framework should be appropriate scaffold for developing novel FtsZ inhibitors. To obtain a series of trisubstituted benzimidazole (BAZs) as the basic scaffold for the novel benzimidazole based compounds, 2,5,6-trisubstituted benzimidazole intermediates having different R groups were synthesized.

According to the previous study in the Ojima group, overexpression of FtsZ was performed by cloning the WT-FtsZ into pET15b vector (Novagen) with a *N*-terminal six-His tag. Purification of FtsZ was carried out by SDS-PAGE as the Ni_{2+} NTA affinity chromatography which provided FtsZ with relatively high purity (**Figure 2-2**). The protein was immediately loaded onto G-25 size exclusion chromatography after elution from the affinity resin by imidazole solution. The fraction from the peak were pooled, concentrated and stored in aliquots at -80 °C. A typical yield from a 1-liter culture was 15 mg of FtsZ. The concentration of the protein was determined using BCA kit from

Sigma, since FtsZ only contains 1 Tyr and no Trp residues. The theoretical molecular weight of FtsZ was 39,578.7 Da, with 285 amino acids.

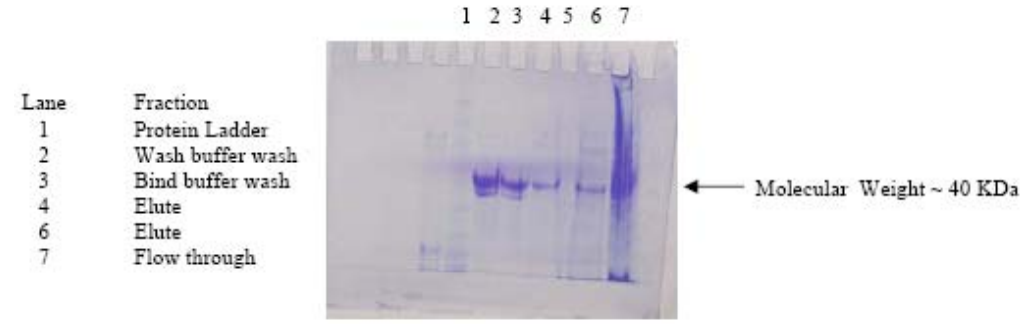


Figure 2-2: SDS-PAGE Gel of WT-FtsZ Preparation

For further investigation, light scattering assay was used to study FtsZ polymerization and used to determine the minimum concentration of the protein required for polymerization. FtsZ (10-15 µM) was incubated at room temperature in polymerization buffer. An immediate increase in light scattering was observed upon addition of 5 mM GTP, reaching a plateau in about 3 min. (**Figure 2-3**). Depolymerization occurred slowly, taking 1 h or longer. The value of the critical concentration required for maximum polymerization was determined to be 15 µM.

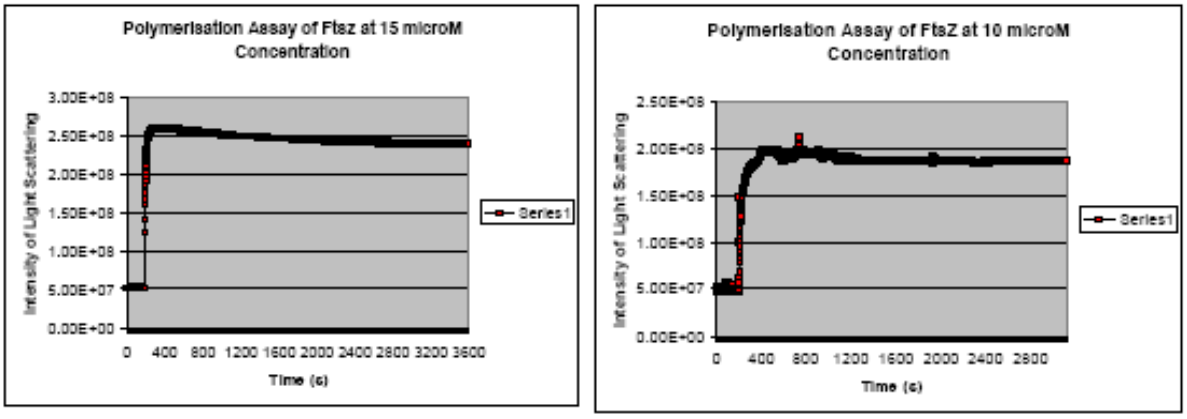


Figure 2-3: Polymerization assay with different concentrations of FtsZ (Wt)

A library of 349 benzimidazole compounds were synthesized. Through preliminary screening against Mtb H37RV cells (drug sensitive Mtb strain), 26 compounds exhibited moderate MIC_{99} activities. Amount these, 8 crude compounds demonstrated MIC_{99} <6 µg/mL and were tested positive for antibacterial activities after

they were re-synthesized and reconfirmed at Dr. Richard Slayden's laboratory at Colorado State University.

Out of the 8 lead compounds, 4 of the trisubstituted benzimidazoles **SB-P3G5**, **SB-P3G2**, **SB-P1G8** and **SB-P1G2** (**Figure 2-4**) were selected to carry out polymerization assay on FtsZ. The assay confirmed that the degree of FtsZ polymerization inhibition corresponded to the varying concentration amounts of benzimidazole. For **SB-P3G5**, 50% inhibition was observed at 10-20 µM concentration and the polymerization was completely shutdown at 40 µM concentration (Figure 4). In the case of **SB-P1G2**, 50% inhibition was observed at 20 µM concentration. However, there was no significant inhibition at 10 µM concentration (**Figure 2-5**). For **SB-P3G2** and **SB-P1G8**, similar patterns in inhibition were observed (**Figure 2-5**), i.e., 50% inhibition at 10 µM concentration and polymerization was completely shut down at 20 µM concentration. The polymerization assay confirmed that benzimidazoles control the bacterial (Mtb) growth by inhibiting FtsZ polymerization.

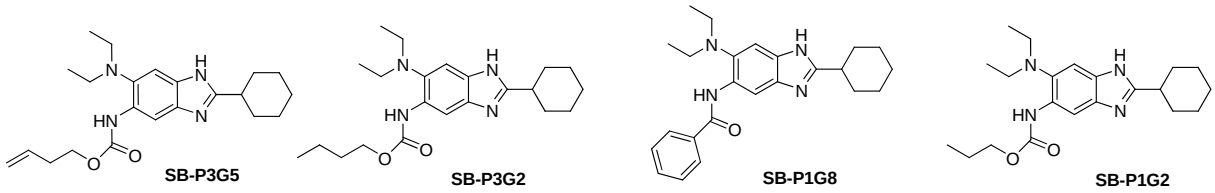
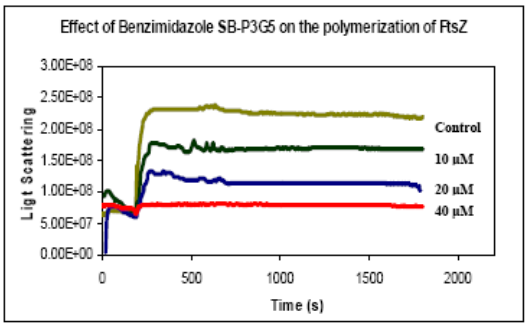
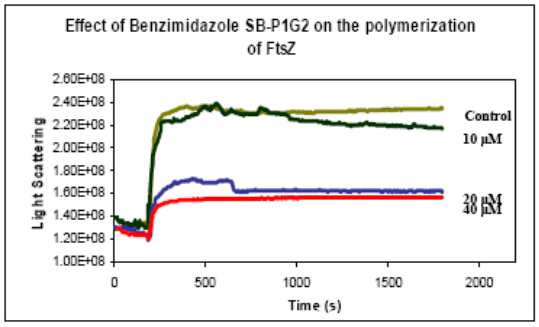


Figure 2-4: Lead Benzimidazole Compounds





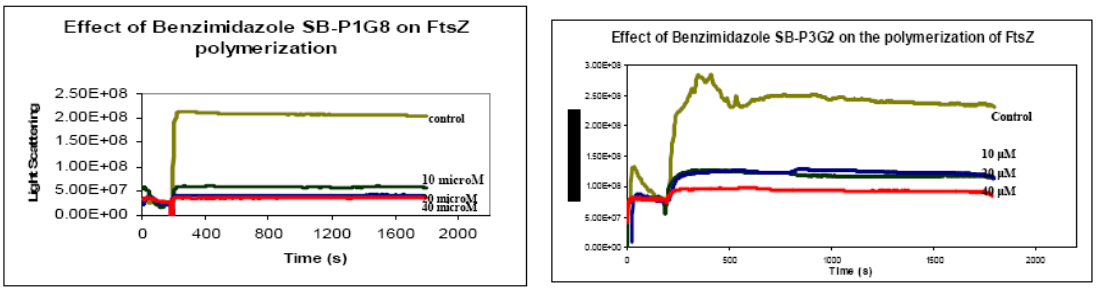


Figure 2-5: Effect of Benzimidazoles on FtsZ Polymerization

2.1.3 Broad-Spectrum Antibacterial Agents

Based on the structural and functional homology of FtsZ and tubulin, it is proposed that compounds which inhibit the (de)polymerization of microtubules should be appropriate lead compounds for discovering novel inhibitors of FtsZ (de)polymerization. As FtsZ is highly conserved protein in many bacterial strains including Vancomycin resistant *Enterococci* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), *staphylococcus aureus*, *Enterococcus faecalis*, *Haemophilus influenza*, it is hypothesized that FtsZ-inhibitors can be developed into broad-spectrum antibacterial agents with novel mechanism of action. Novel polymer-assisted solution phase methods was developed for the synthesis of a library of the first-generation 2,5,6- and 2,5,7- trisubstituted benzimidazoles. As mentioned above, a number of compounds show good activity Hence, optimization of trisubstituted benzimidazole can be done by synthesizing a more diverse benzimidazole library.

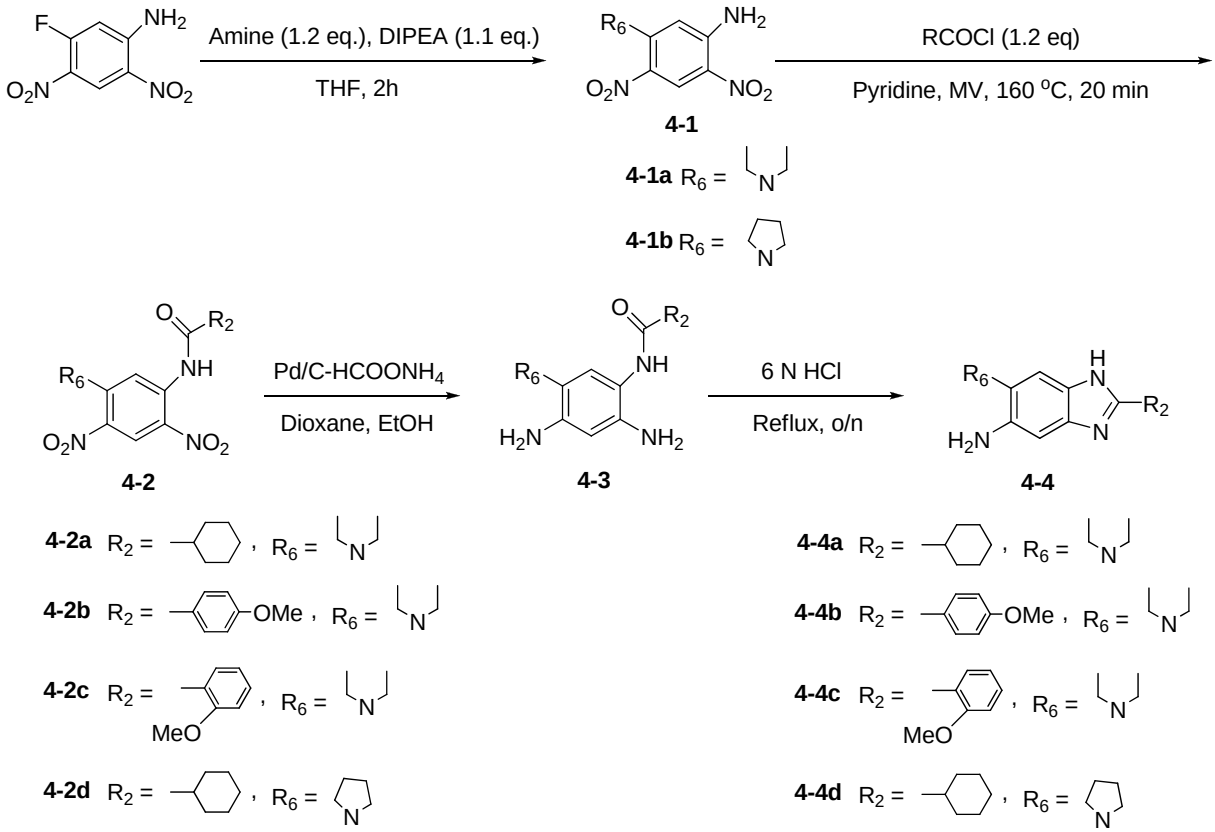
Having core structures defined, the structure –activity relationship (SAR) studies have been performed. To optimize the whole structure, a library of benzimidazoles need to be created, from which, new drug candidates would be discovered.

2.2. Results and Discussion

2.2.1 Synthesis of 2,5,6-trisubstituted benzimidazole intermediates for library synthesis

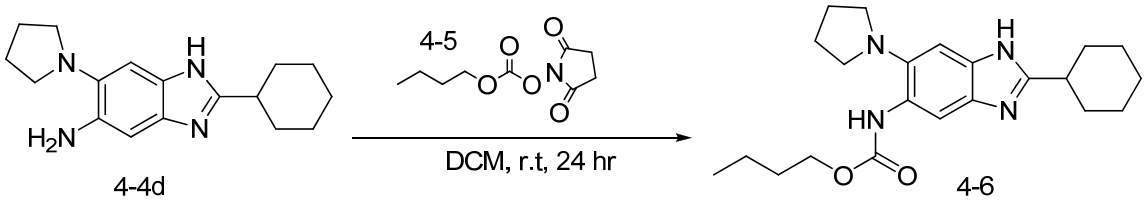
Starting material, 2,4-dinitro-5-fluoroaniline underwent aromatic nucleophilic substitution with an amine to yield **4-1**. The acylation of **4-1** with an acid chloride generated acylated intermediate, **4-2**. Next, the reduction of **4-2** to **4-3** and the subsequent

treatment of **4-3** with hydrochloric acid afforded benzimidazole intermediate **4-4** **(Scheme 2-1)**.



Scheme 2-1: Synthesis of 2,5,6-trisubstituted benzimidazole intermediates

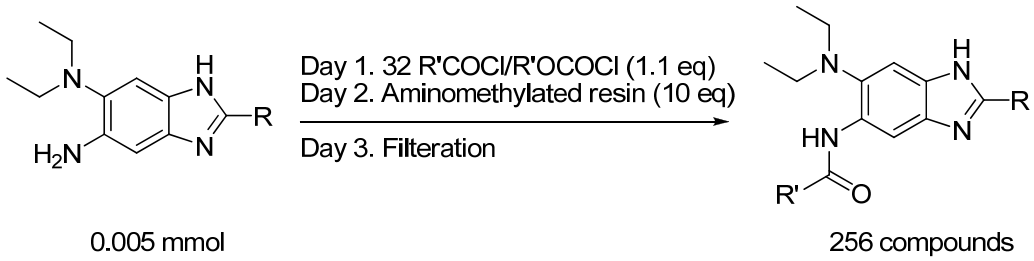
In the past, the acylation of the free 5-amino group of the intermediate **4-4d** in the last step was undertaken by employing buty chloroformate. However, the yield of the desired product was poor because of the occurrence of diacylation. To optimize the reaction, *N*-hydroxysuccinimide was used to make an active ester, which increased the reacting rate significantly and reduced the formation of bi-product (i.e., diacylation product). In each step, no column purification was necessary, wherein recrystallization was used instead (**Scheme 2-2**).



Scheme 2-2: Synthesis of 2,5,6-trisubstituted benzimidazole **4-6**

2.2.2 Synthesis of trisubstituted benzimidazole library with diethyl amino moiety at C-6 position.

A library synthesis was performed by using 96 well plates (**Scheme 2-3)**. Eight 2,5,6 trisubstituted intermediates (0.005 mM) (**Figure 2-6**) were dissolved in dichloromethane and transferred into the 96 well plates (Column A-H). Next, 33 different acylating reagents (acid chlorides (1.1 eq) and chloroformates (0.9 eq)) were added to each individual well. Total of three 96 well plates were used, with 11 acylating reagents in each row (**Figure 2-7**). The plates were slowly shaken for overnight. Aminomethylated resins (10 eq) were introduced to scavenge excess reagents in the following day and were stirred for overnight. Resins were washed with dichloromethane and filtered to produce the library of trisubstituted benzimidazoles on day 3.



Scheme 2-3: Synthesis of 2,5,6-diethylamino library

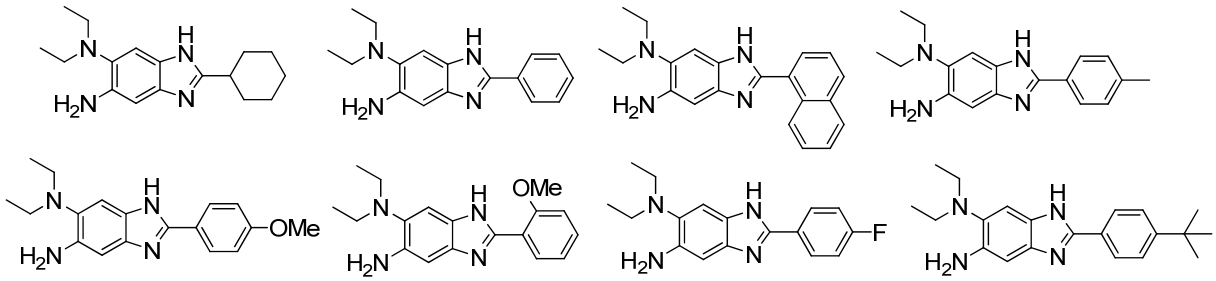


Figure 2-6: Trisubstituted intermediates used in synthesis of 2,5,6-diethylamino library

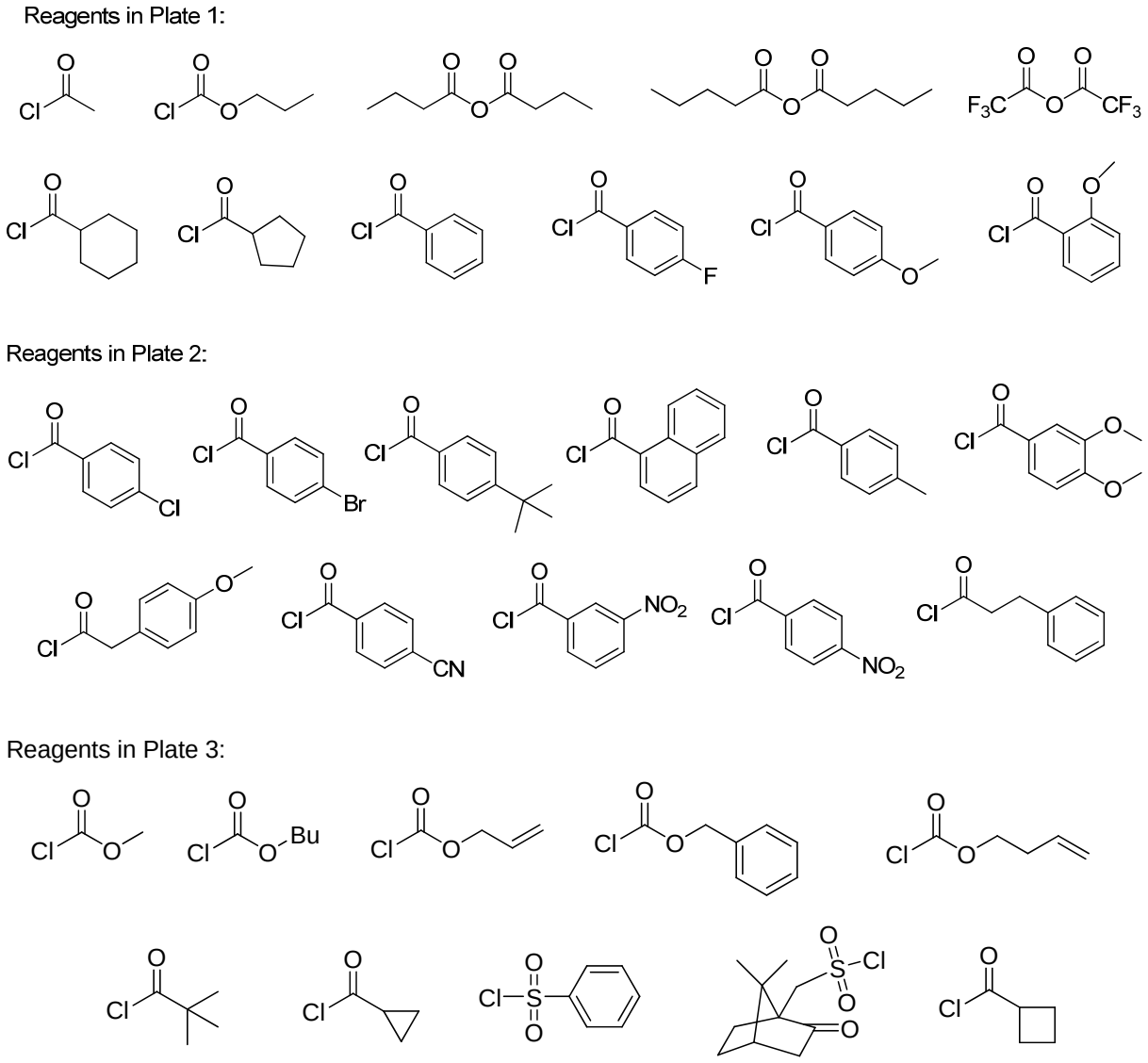


Figure 2-7: Reagents used in synthesis of diethylamino library

The purpose of the combinatorial chemistry synthesis which was designed by the Ojima group in the past was to optimize micro scale synthesis in 96 well plates. However, based on the experimental results and analysis by liquid chromatography – mass spectrometry (LC/MS), it seems that an alternative synthesis method needs to be designed to improve purity of the desired product. Among the 256 reactions, only a few had completed its reaction, majority of them still contained unreacted starting material.

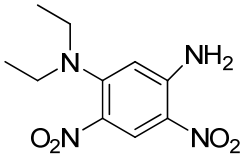
General Method and Materials:

NMR spectra were measured on a Bruker AC-25- NMR spectrometer or a Varian 300 NMR spectrometer using tetramethylsilane as the internal standard. Melting point was measured on a Thomas Hoover Capillary melting point apparatus. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter. TLC was performed on Merck DC-alufolien with Kieselgel 60F-254 and column chromatography was carried out on silica gel 60(Merck; 230-400 mesh ASTM). Chiral HPLC analysis for the determination of enantiomeric excess was carried out with a Waters HPLC assembly. HPLC assembly comprises Waters M45 solvent delivery system, Waters Model 680 gradient controller, Water M440 detector (at 254 nm) equipped with a Spectra Physics Model SP4270 integrator. The system uses a DAICEL-CHIRACEL OD chiral column (25 x 0.46 cm i.d.), employing hexane/2-propanol (99.5/0.5, v/v) as the solvent system with a flow rate of 1.0 ml/min. HPLC analysis for determination of isomeric ratio was carried out with the same Water HPLC assembly using 5u Spherical Silica column employing hexane/2-propanol/dichloromethane (15/1/1, v/v/v) as the solvent system with a flow rate 1.0 ml/min, or hexane/2-propanol/dichloromethane (10/1/1, v/v/v) as the solvent system with a flow rate 1.4 ml/min. Elemental analysis were performed at M-H-W Laboratory, Phoenix, AZ. High resolution mass spectra were obtained from the California, Riverside Mass Spectrometry Facility, Riverside, CA; or Mass Spectrometry Laboratory, University of Illinois at Urbana-Champaign, Urbana, IL.

The chemicals were purchased from Aldrich and Sigma and purified before use by standard methods. Dichloromethane was distilled under nitrogen from calcium hydride immediately before use.

2.3. Experimental Section

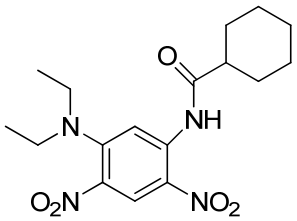
5-*N*,*N*-Diethylamino-2,4-dinitroaniline (4-1a) (53)



One equivalent of 2,4-dinitro-5-fluoroaniline (1.00 g, 4.97 mmol) and 1.1 equivalents of diisopropylethylamine (0.95 mL) were dissolved in THF (50 mL). Then, 1.2 equivalents

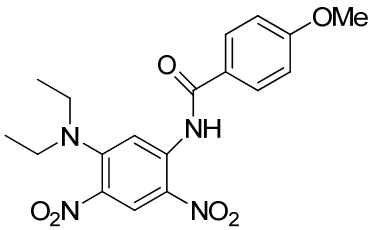
of diethylamine (0.63 mL) was dissolved in THF (50 mL) and added to the first solution, dropwise. The reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated and once its dried, dichloromethane and water were added for extraction. The organic layer was collected, filtered and concentrated under pressure to give the desired product **4-1a (**1.17 g, 83 % yield) as yellow solid: ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.76 (s, 1 H) 6.11 (s, 1 H), 3.27 (q, 4 H, *J* = 7.2 Hz), 1.20 (t, 6 H, *J* = 6.9); ESI MS m/z 255.1 $[M+H]^+$.

***N*-(5-*N*-*N*-Diethylamino-2,4-dinitrophenyl) cyclohexanecarboxamide (4-2a)** (53)



The obtained **4-1a** (1.05 g, 4.13 mmol) was mixed with pyridine (8 mL) and cyclohexanecarbonyl chloride (0.67 mL). The mixture was placed into a microwave (MW) reactor for 20 min at 160 °C. The reaction was monitored *via* TLC. The reaction mixture was diluted with dichloromethane, washed with water for three times, dried over magnesium sulfate and concentrated *in vacuo*. The resulting residues was separated and purified by flash chromatography on silica gel using hexane:ethyl acetate (9:1) as the eluent to yield **4-2a (**1.17 g, 83 %) as yellow solid: ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.76 (s, 1 H), 8.65 (s,1 H), 3.36 (q, 4 H, *J* = 10.8 Hz), 2.38 – 1.30 (m, 11 H), 1.26 (t, 6 H, *J* = 7.2 Hz); ESI MS m/z 365.4 $[M+H]^+$.

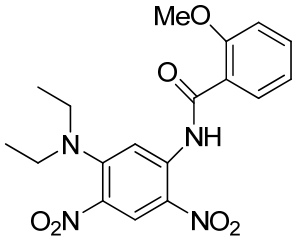
***N*-(5-*N*-*N*-Diethylamino-2,4-dinitrophenyl)-4-methoxybenzamide (4-2b)** (53)



The obtained **4-1b** (969 mg, 3.81 mmol) was mixed with pyridine (8 mL) and 4-methoxybenzoyl chloride (0.62 mL). The solution mixture was placed into a MW reactor for 20 min at 160 °C.

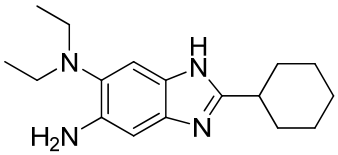
The reaction was monitored by TLC. The reaction mixture was diluted with dichloromethane, washed with water for three times, dried over magnesium sulfate and concentrated *in vacuo*. The resulting residues was separated and purified by flash chromatography on a silica gel using hexane:ethyl acetate (9:1) as the eluent to yield **4-2b** (820 mg, 55 %) as yellow solid: ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.94 (s, 1 H), 8.77 (s, 1 H), 8.18 (d, 1 H, J = 4.6 Hz), 7.51 (m, 1 H), 7.08 (t, 1 H, J = 7.6 Hz), 7.02 (d, 2 H, J = 4.2 Hz), 4.08 (s, 3 H), 3.37 (q, 4 H, J = 10.6 Hz), 1.27 (t, 6 H, J = 7.2 Hz); ESI MS m/z 389.3 $[M+H]^+$.

***N*-(5-*N*-*N*-Diethylamino)-2,4-dinitrophenyl)-2-methoxybenzamide (4-2c)** (53)



The obtained **4-1c** (1.01 g, 3.93 mmol) was mixed with pyridine (8 mL) and 2-methoxybenzoyl chloride (0.64 mL). The solution mixture was placed into a MW reactor for 20 min at 160 °C. The reaction was monitored by TLC. The reaction mixture was diluted with dichloromethane, washed with water for three times, dried over magnesium sulfate and concentrated *in vacuo*. The resulting residues was separated and purified by flash chromatography on a silica gel using hexane:ethyl acetate (9:1) as the eluent to yield **4-2c** (1.47 g 96 %) as yellow solid: ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.94 (s, 1 H), 8.77 (s, 1 H), 8.18 (d, 1 H, J = 4.6 Hz), 7.51 (m, 1 H), 7.08 (t, 1 H, J = 7.6 Hz), 7.02 (d, 2 H, J = 4.2 Hz), 4.08 (s, 3 H), 3.37 (q, 4 H, J = 10.6 Hz), 1.27 (t, 6 H, J = 7.2 Hz); ESI MS m/z 389.3 $[M+H]^+$.

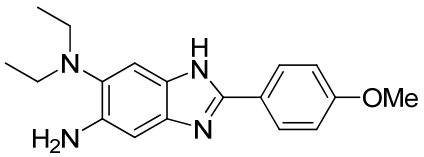
5-Amino-2-cyclohexyl-*N*,*N*-diethylaminobenzimidazole (4-4a)



The obtained **4-2a (**1.32 g, 3.63 mmol), was dissolved in dioxane (45 mL) and ethanol (45 mL). Ammonium formate (5.26 g, 86.1 mmol) and 10 % Pd-C (400 mg, 3.76 mmol) was added into the solution and the mixture was stirred for 1 h at room temperature. The

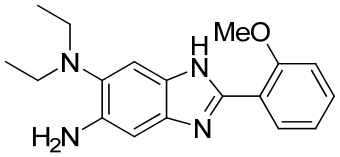
catalyst and excess ammonium formate was filtered to obtain a solution of **4-3** in dioxane and ethanol. Concentrated aqueous solution of hydrochloric acid was added to the filterate to make it 6 M HCl and refluxed for 3 h. The reaction mixture was basified by adding ammonium hydroxide, extracted with ethyl acetate three times, dried over magnesium sulfate and concentrated *in vacuo*. The resulting residues was separated and purified by flash chromatography on silica gel using hexane:ethyl acetate (1:1) as the eluent to yield **4-4a** (472 mg, 45 % in two steps) as brown solid: ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.31 (s, 1 H), 6.9 (s, 1 H), 2.92 (m, 4 H, *J* = 10.8 Hz), 2.04 (m, 2 H), 1.68 (m, 5 H), 1.26 (m, 4 H), 0.95 (t, 6 H, *J* = 6.9 Hz); ESI MS m/z 287.4 $[M+H]^+$.

5-Amino-6-*N*,*N*-diethylamino-2-(4-methoxyphenyl)benzimidazole (4-4b) (53)



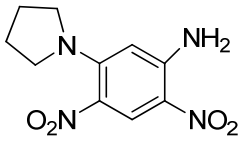
The obtained **4-2b (**744 mg, 1.91 mmol**)** was dissolved in dioxane (45 mL) and ethanol (45 mL). Ammonium formate (2.87 g, 45.5 mmol) and 10 % Pd-C (373 mg, 3.50 mmol) was added into the solution and the mixture was stirred for 1 h at room temperature. The catalyst and excess ammonium formate was filtered to obtain a solution of **4-3** in dioxane and ethanol. Concentrated aqueous solution of hydrochloric acid was added to the filterate to make it 6 M HCl and refluxed for 3 h. The reaction mixture was basified by adding ammonium hydroxide, extracted with ethyl acetate three times, dried over magnesium sulfate and concentrated *in vacuo*. The resulting residues was separated and purified by flash chromatography on silica gel using hexane:ethyl acetate (1:1) as the eluent to yield **4-4b** (350 mg, 59 % in two steps) as brown solid: ^{1}H-NMR (300MHz, $CDCl_3$) δ 0.93 (t, 6H, J = 7.2 Hz), 2.92 (q, 4H, J = 7.2 Hz), 4.03 (s, 3H), 6.73 (s, 1H), 6.75 (d, 2H, J = 8.4 Hz), 7.10 (d, 2H, J = 8.4 Hz) 7.16 (s, 1H); ESI MS m/z 311.1 [M+H]+.

5-Amino-6-*N*,*N*-diethylamino-2-(2-methoxyphenyl)benzimidazole (4-4c) (53)



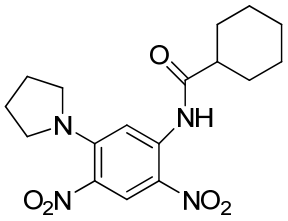
The obtained **4-2c (**1.23 g, 3.17 mmol**)** was dissolved in dioxane (45 mL) and ethanol (45 mL). Ammonium formate (8.475 g, 134 mmol) and 10 % Pd-C (738 mg, 6.93 mmol) was added into the solution and the mixture was stirred for 1 h at room temperature. The catalyst and excess ammonium formate was filtered to obtain a solution of **4-3** in dioxane and ethanol. Concentrated aqueous solution of hydrochloric acid was added to the filterate to make it 6 M HCl and refluxed for 3 h. The reaction mixture was basified by adding ammonium hydroxide, extracted with ethyl acetate three times, dried over magnesium sulfate and concentrated *in vacuo*. The resulting residues was separated and purified by flash chromatography on silica gel using hexane:ethyl acetate (1:1) as the eluent to yield **4-4c** as in 57 % (two steps) as brown solid:^{1}HNMR (400 MHz, $CDCl_3$) δ 8.48 (dd, 1 H), 7.37-7.29 (m, 2 H), 7.48 (t, 1 H, *J* = 8 Hz), 6.97 (d , 1 H, *J* = 4.2 Hz), 6.88 (s, 1 H), 3.96 (s, 3 H), 2.96 (q, 4 H, *J* = 10.6 Hz), 0.97 (t, 6 H, *J* = 7 Hz); ESI MS m/z 311.3 $[M+H]^+$

Pyrrolidin-2,4-5-(pyrrolidin-1-yl)dinitroaniline (4-1b) (53)



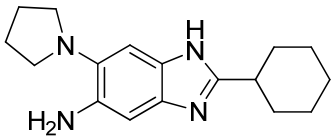
One equivalent of 2,4-dinitro-5-fluoroaniline (5.10 g, 25.4 mmol) was first dissolved in THF (250 mL). Then, 1.1 equivalents of diisopropylethylamine (4.8 mL) was added followed by 1.2 equivalents of pyrrolidine (2.5 mL) in THF (250 mL) , dropwise. The reaction mixture was stirred at room temperature for 2 h. Once the reaction was complete, the solvent was evaporated and ethyl acetate was added. The organic layer was separated, washed with water and brain and dried over sodium sulfate. The solvent was removed under reduced pressure to give crude **4-1b** as yellow solid (6.01 g, 94 %)**:** mp 170-171 ºC (Literature value: 169-171 ºC);^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05-2.12 (m, 4 H), δ 3.35 (t, 4 H, J = 6.6 Hz), δ 5.98 (s, 1 H), δ 6.37 (s, 2 H), δ 8.81 (s, 1 H).

***N*-2,4-Dinitro-5-(pyrrolidin-l-yl)phenyl cyclohexanecarboxamide (4-2d)** (53)



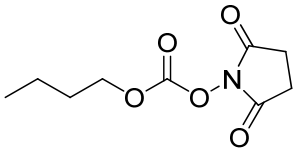
The obtained **4-1b (**2.00 g, 7.92 mmol) was dissolved in pyridine (16 mL). Then, 1.5 equivalents of cyclohexanecarbonyl chloride (1.5 mL) was added dropwise, and the resulting solution was placed in a microwave reactor for 50 min at 100 °C. The reaction was monitored by TLC. When the reaction was complete, the reaction mixture was poured into a beaker with 250 mL cold water with stirring to form precipitant. A crude yellow solid **4-2d** was obtained by filtration in quantitative yield. It was pure enough for the next reaction: mp 183-184 °C (Literature value: 181-182 ºC); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.56-2.05 (m, 7 H), 3.37 (t, 4 H, J = 6.6 Hz), 8.51 (s, 1 H), 8.80 (s, 1 H).

5-Amino-2-cyclohexyl-6-pyrrolidin-1-ylbenzimidazole (4-4d)



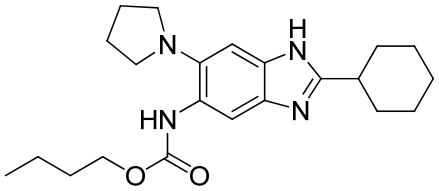
The obtained **4-2d (**5.00 g, 13.8 mmol) was dissolved in dioxane (170 mL) and ethanol (170 mL). Ammonium formate (23.6 g) and 10 % Pd-C (3.07 g) were added into the solution and the mixture was stirred for 1 h at room temperature. The catalyst and excess ammonium formate was filtered to obtain the solution of **4-3** in dioxane and ethanol. Concentrated aqueous solution of hydrochloric acid was added to the filtrate to make 6 M of HCl. The reaction mixturewas stirred at room temperature for overnight. Once the reaction was complete (monitoring by flow injection analysis (*FIA*) mass), the reaction mixture was basified with ammonium hydroxide, extracted with ethyl acetate three times, washed with water and brine, dried over magnesium sulfate and concentrated *in vacuo*. The resulting residues was separated and purified by flash chromatography on silica gel using hexane:ethyl acetate (1:1) as the eluent to yield **4-4d** (2.34 g, 60 % in two steps) as brown solid.

2,5-Dioxopyrrolidinyl-n-butylcarbonate (4-5)



To prepare the active ester, 1.2 equivalents of *N*-hydroxysuccinimide was dissolved in dry DCM (10 ml) under nitrogen at 0 °C in an ice bath and 1 equivalent of butyl chloroformate (2.0 ml) was added, followed by 1.5 equivalents of TEA (2.2 mL) in dry DCM (20 mL). Once all reagents were added, additional dry DCM (30 mL) was added and the reaction flask was removed from the ice bath to sit at room temperature overnight. The reaction mixture was diluted with dichloromethane, washed with water for three times, dried over magnesium sulfate and concentrated *in vacuo* to obtain the active ester **4-5** as clear oil in quantitative yield*:* ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.931 (t, J = 7.35, 3H), 1.40 (m, 2H), 1.71 (m, 2H), 2.81 (s, 4H), 4.30 (t, J = 6.45 Hz, 2H)

2-Cyclohexyl-5-butoxycarbonylamino-6-pyrrolidinylbenzimidazole (4-6)



Active ester **4-5** (282 mg, 4.04 mmol) was diluted in DCM (20 ml) and was added into the flask to react with 1 equivalent of **4-4** in DCM (10 mL) at room temperature. When the reaction was complete, the reaction mixture was diluted with DCM and washed with water and brine. The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The resulting residues was separated and purified by flash chromatography on silica gel using hexane:ethyl acetate (9:1) as the eluent to give **4-6** as white solid (305 mg, 80 %): mp 57-59 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, J = 7.0 Hz, 3H), 1.20–2.08 (m, 18H), 2.84 (s, 1H), 2.99 (t, J = 6.6 Hz, 4H), 4.19 (t, J = 6.75 Hz, 2H), 7.48 (s, 1H), 7.90 (s, 1H)

2.4. References

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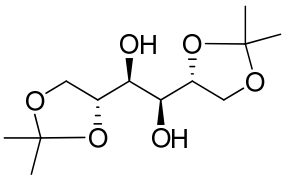
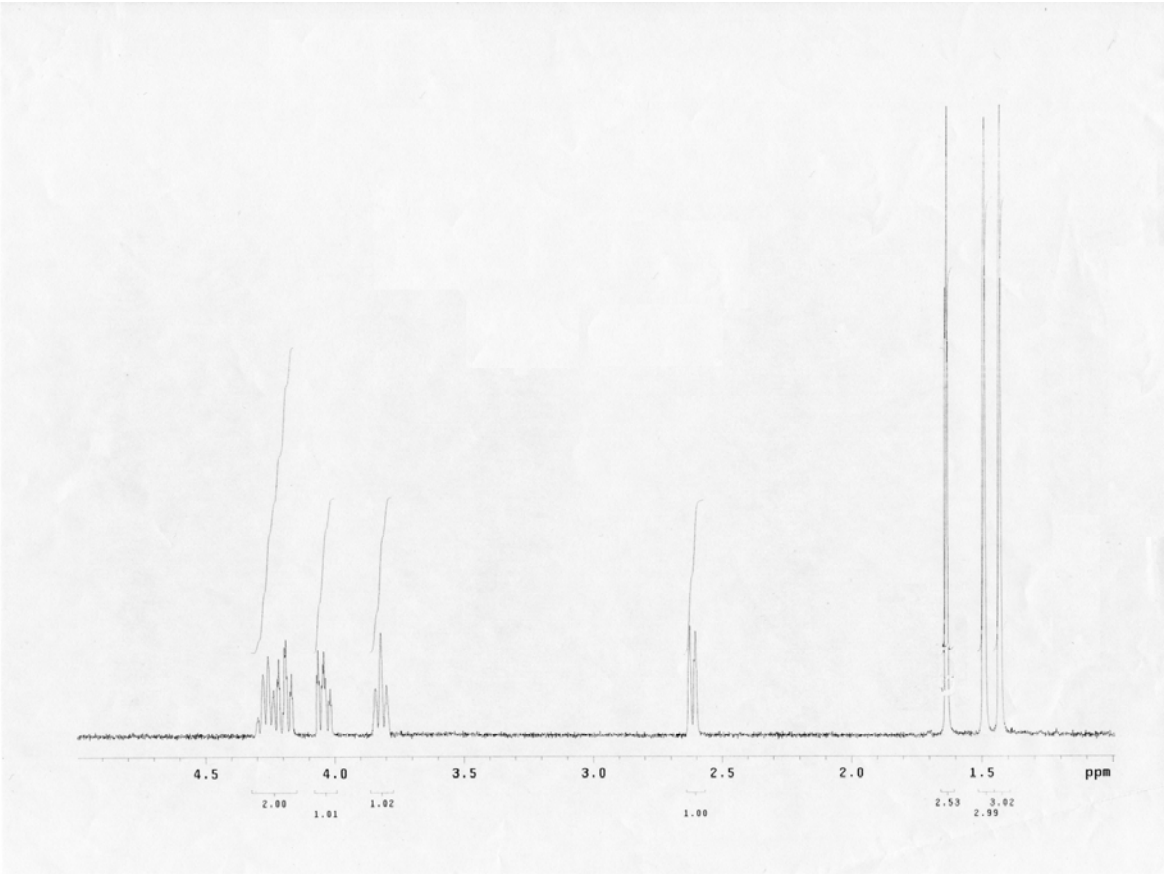
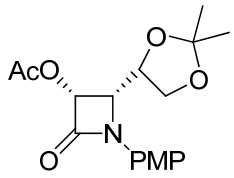
Appendix

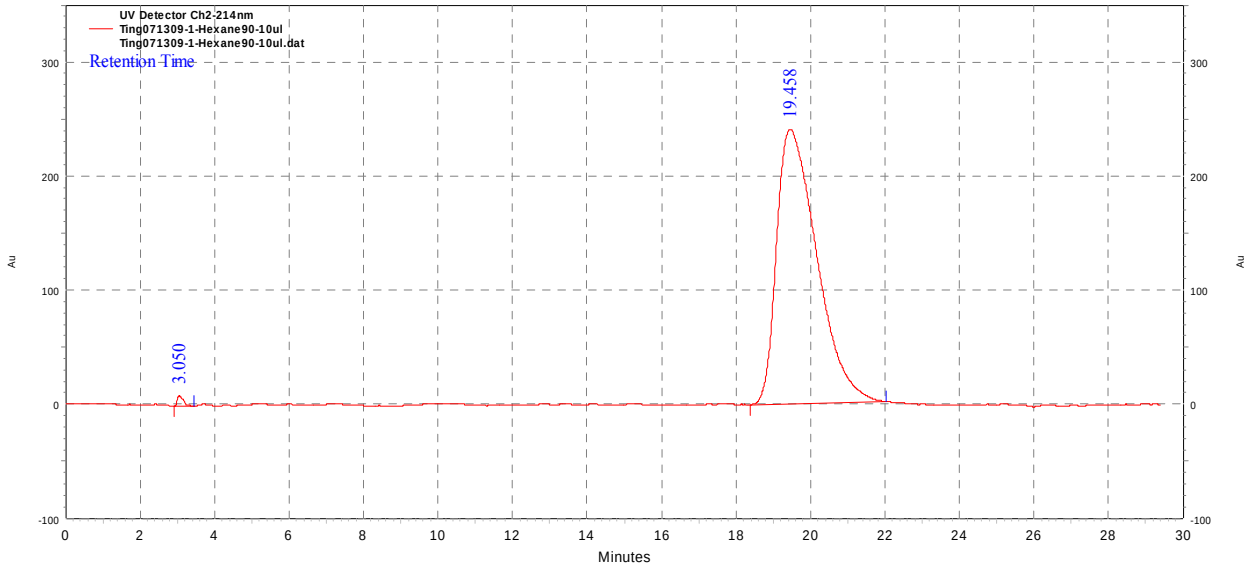
^{1}H NMR Spectrum of **1,2:5,6-Di-O-isopropylidene-D-mannitol (2-1)**

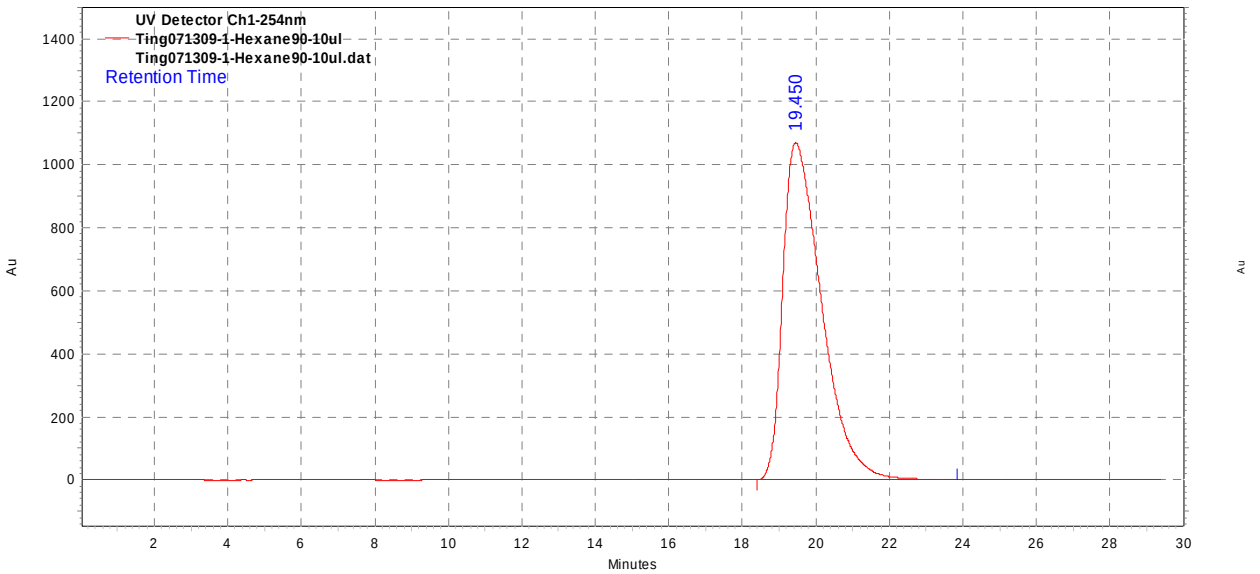




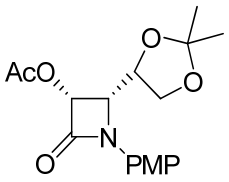
HPLC Spectrum of **1-PMP-(3R)-acetoxy-(4S)-(2,2-dimethyl-1,3-dioxolan-4-yl)-azetidin-2-one (2-3)**

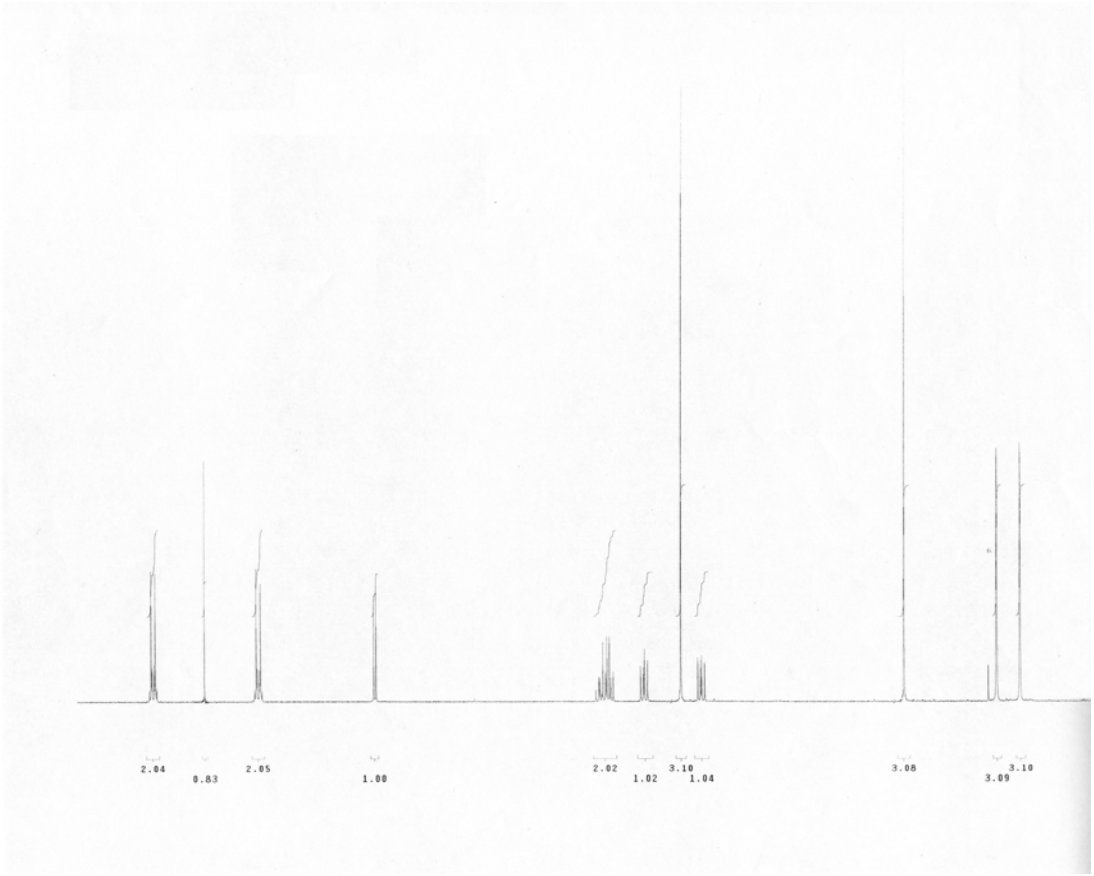




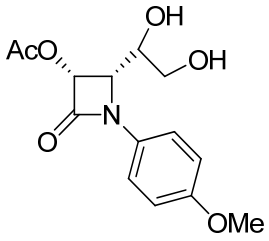


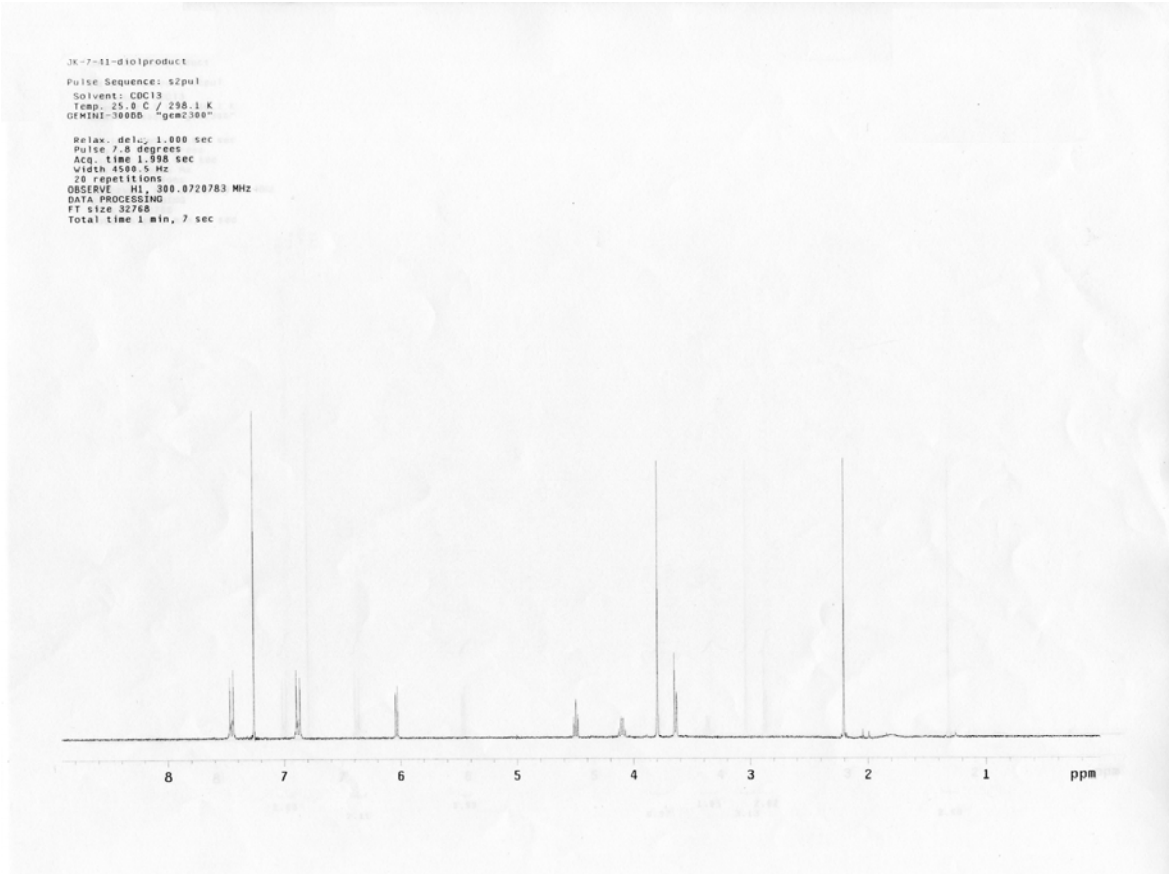
^{1}H NMR Spectrum of **1-PMP-(3*R*)-acetoxy-(4*S*)-(2,2-dimethyl-1,3-dioxolan-4-yl)-azetidin-2-one (2-3)**





^{1}H NMR Spectrum of **1-PMP-(3*R*)-acetoxy-(4*S*)-(1,2-dihydroxy-ethyl)azetidin-2-one (2-4)**





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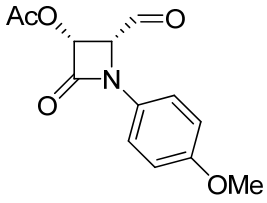
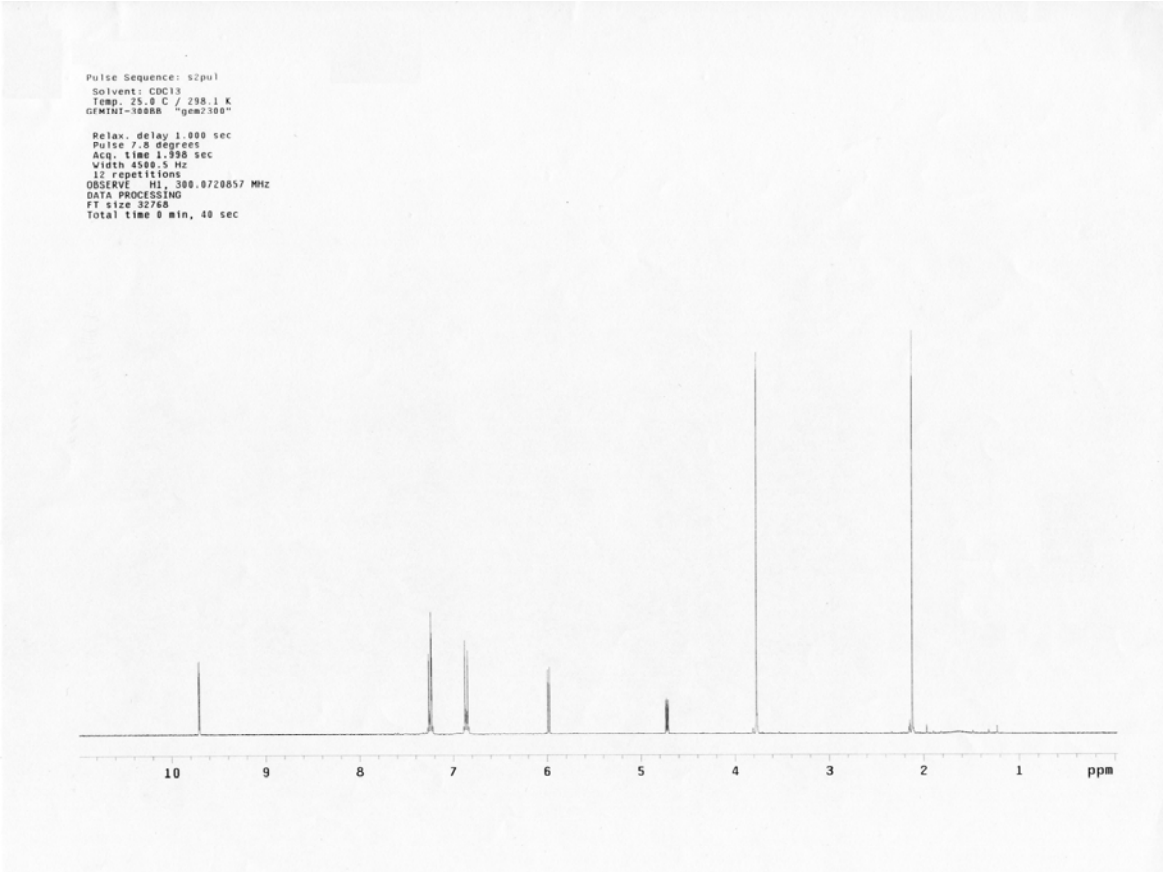
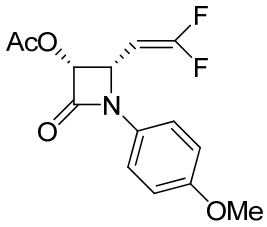
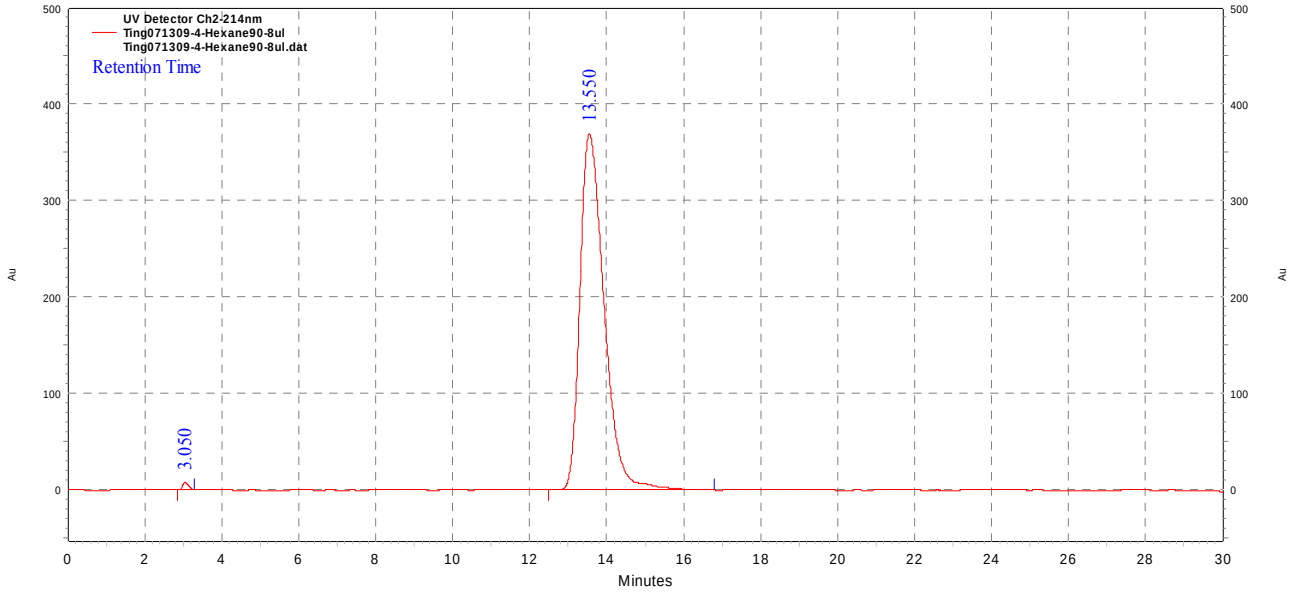
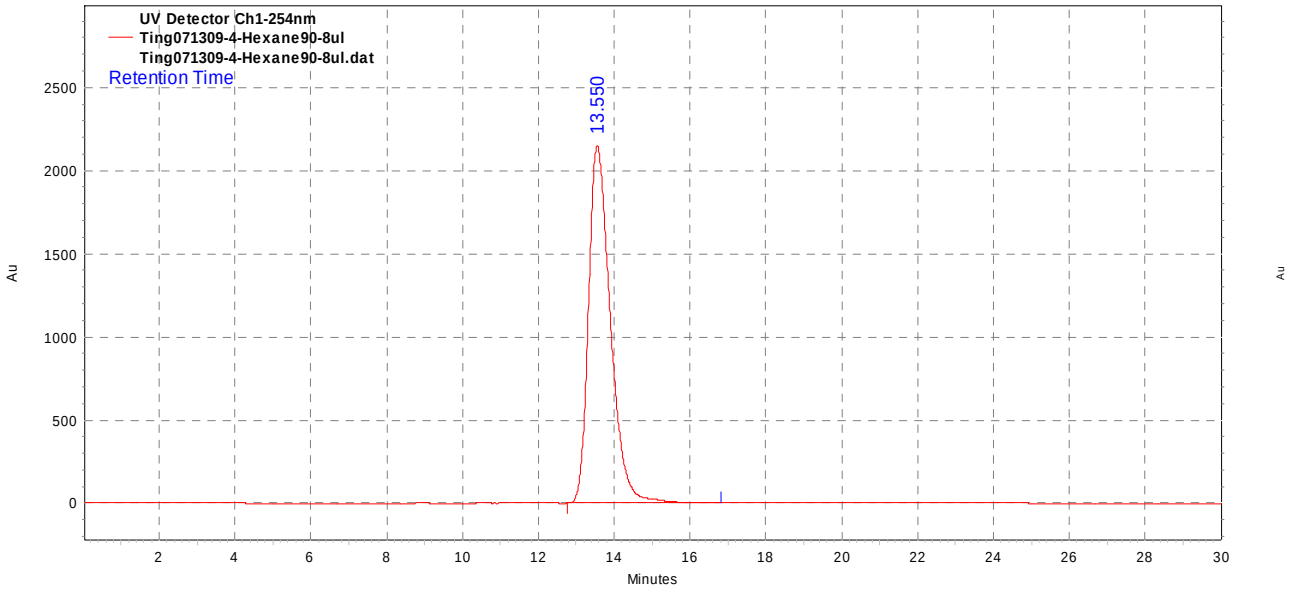




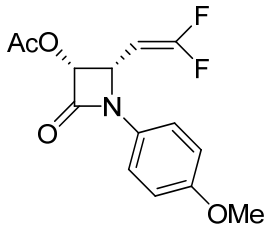
HPLC Spectrum of **1-PMP-(3R)-Acetoxy-(4S)-difluorovinyl-azetidin-2-one (2-6)**

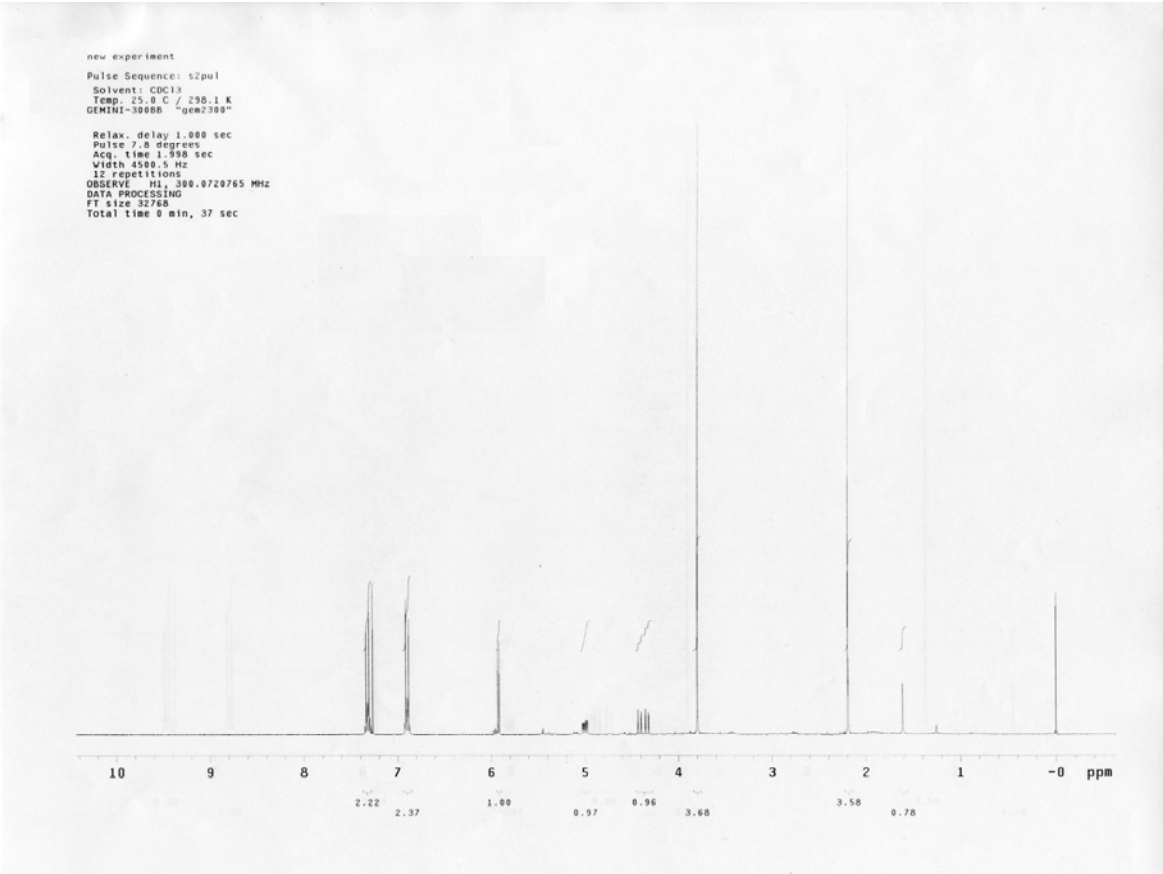




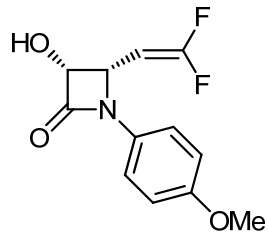


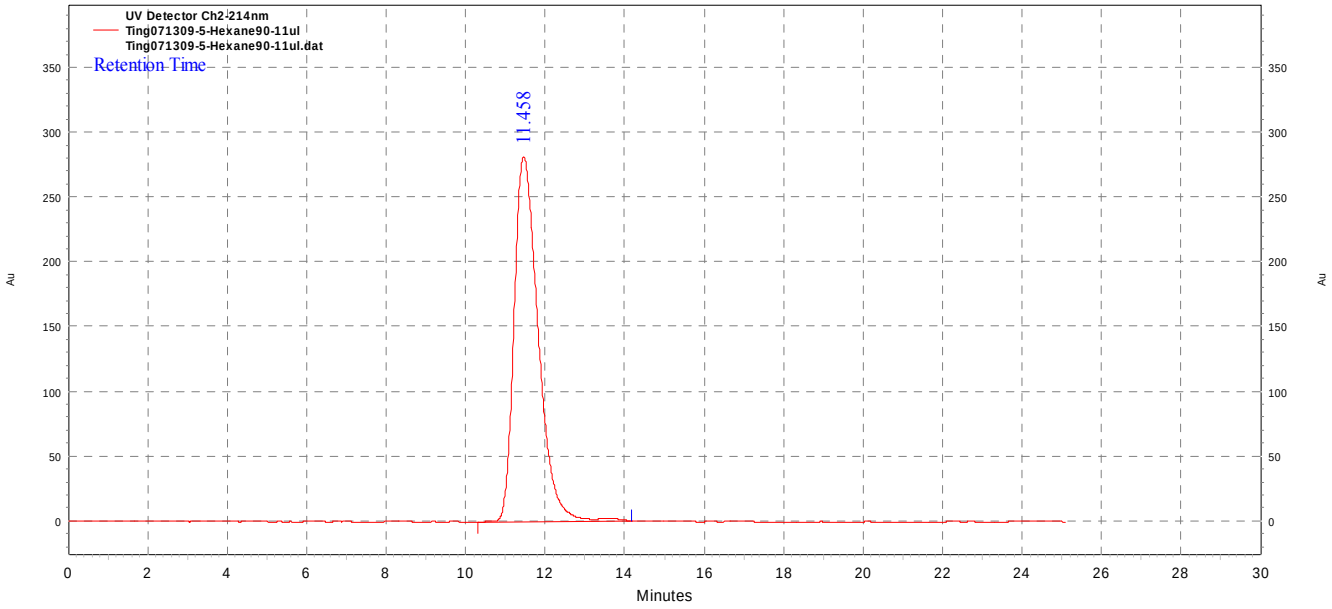
^{1}H NMR Spectrum of **1-PMP-(3*R*)-acetoxy-(4*S*)-difluorovinylazetidin-2-one (2-6)**

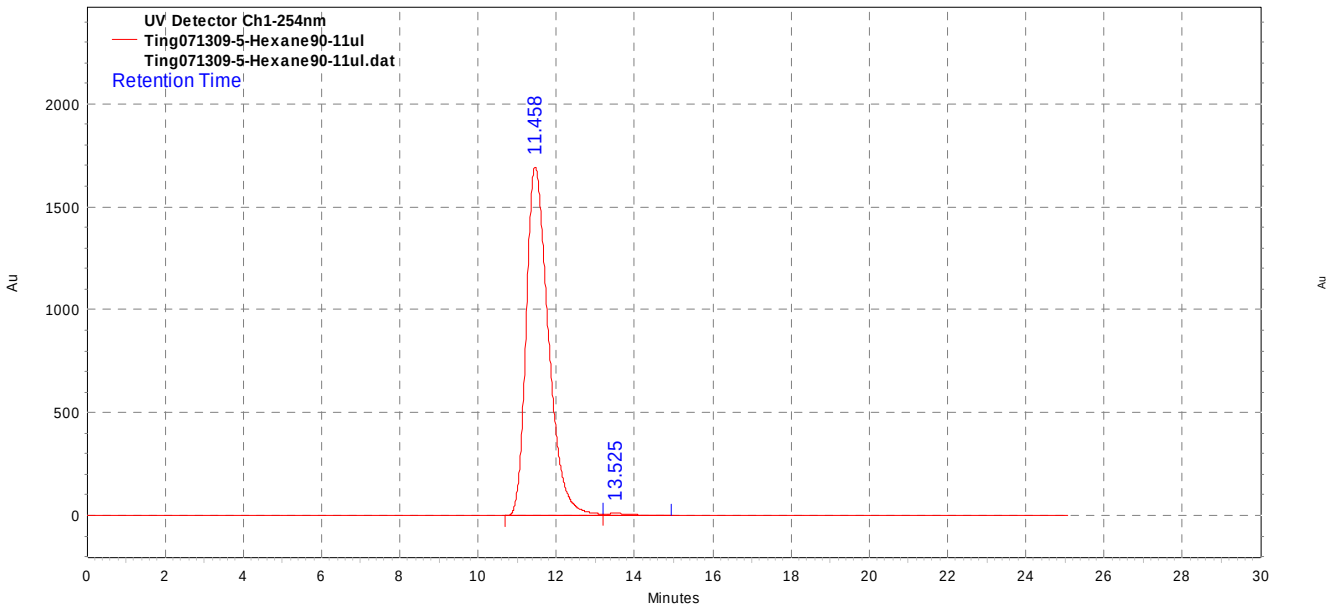




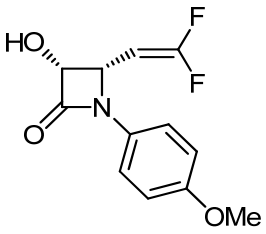
HPLC Spectrum of **1-PMP-(3R)-hydroxyl-(4S)-difluorovinyl-azetidin-2-one (2-7)**

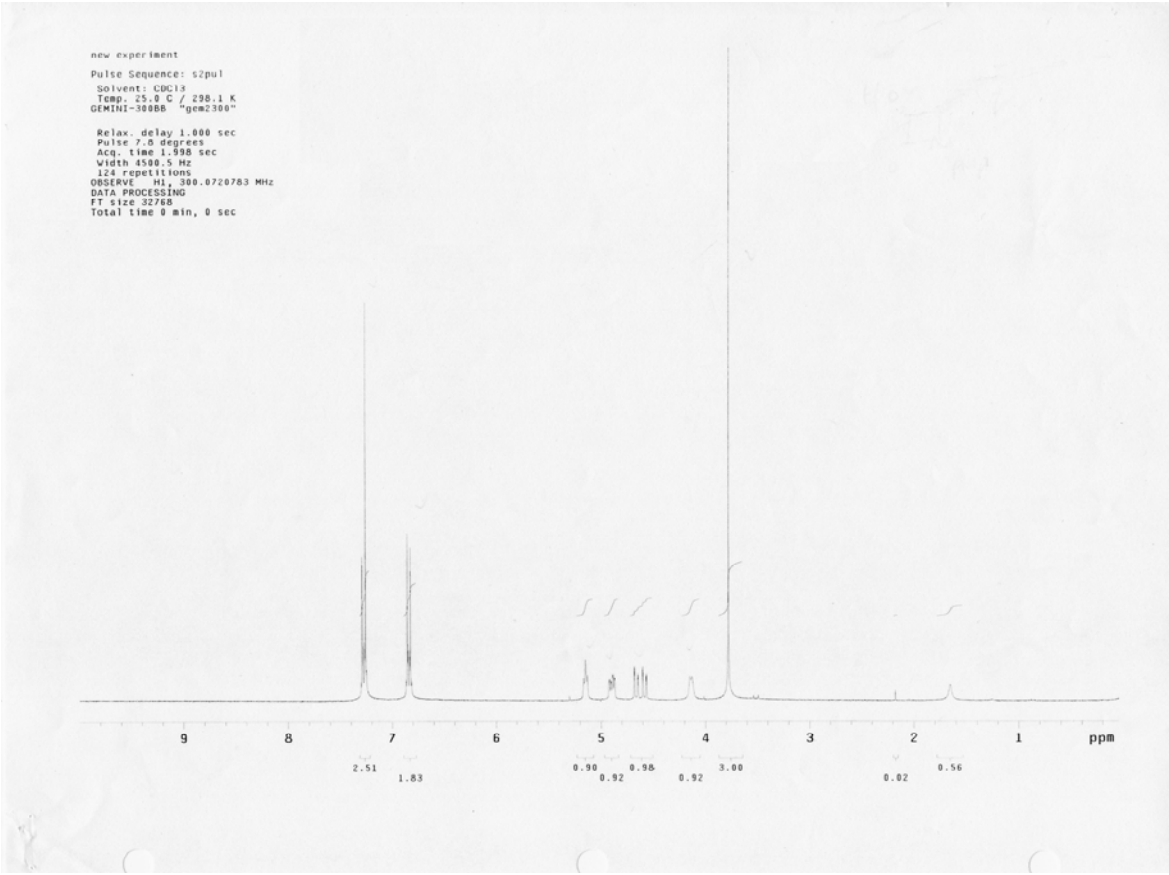




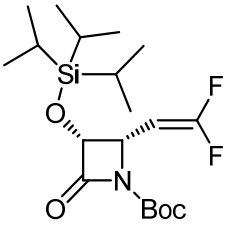


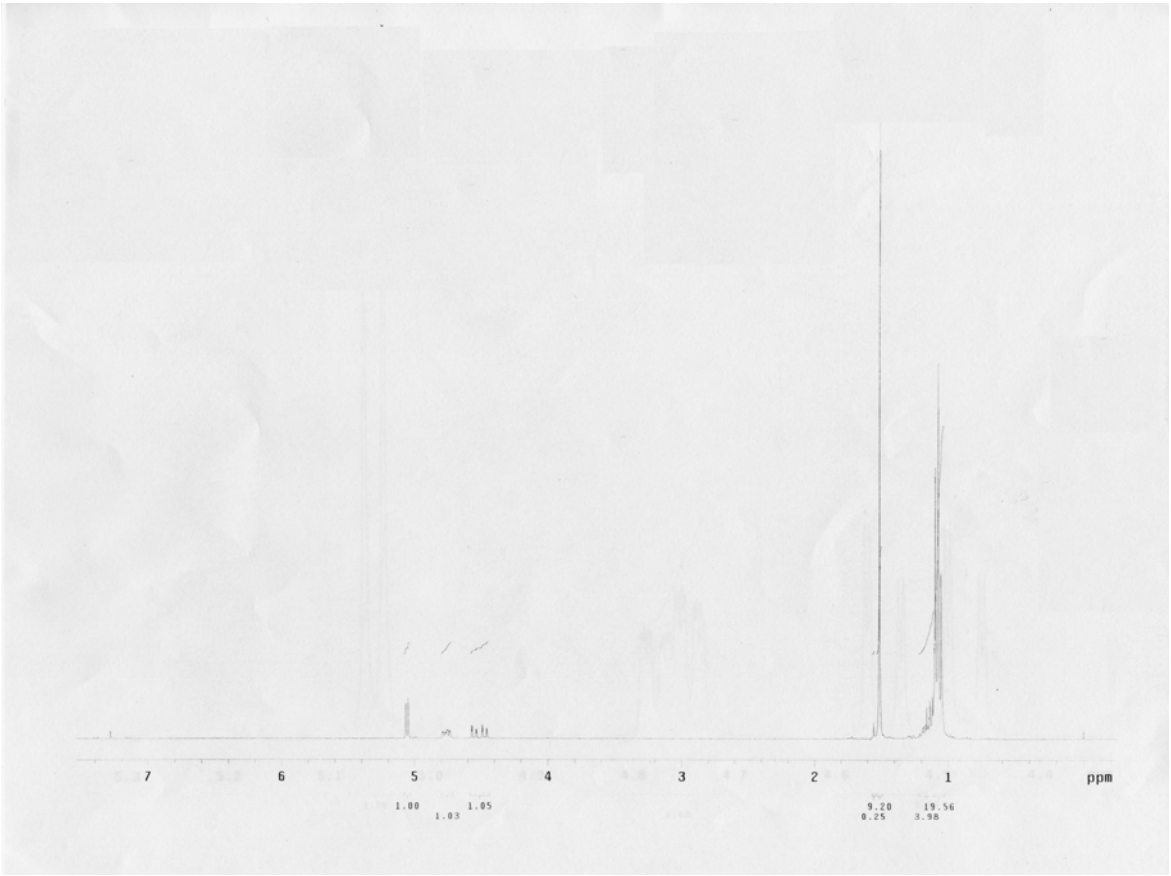
^{1}H NMR Spectrum of **1-PMP-(3*R*)-hydroxyl-(4*S*)-difluorovinylazetidin-2-one (2-7)**



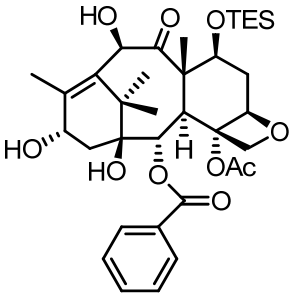


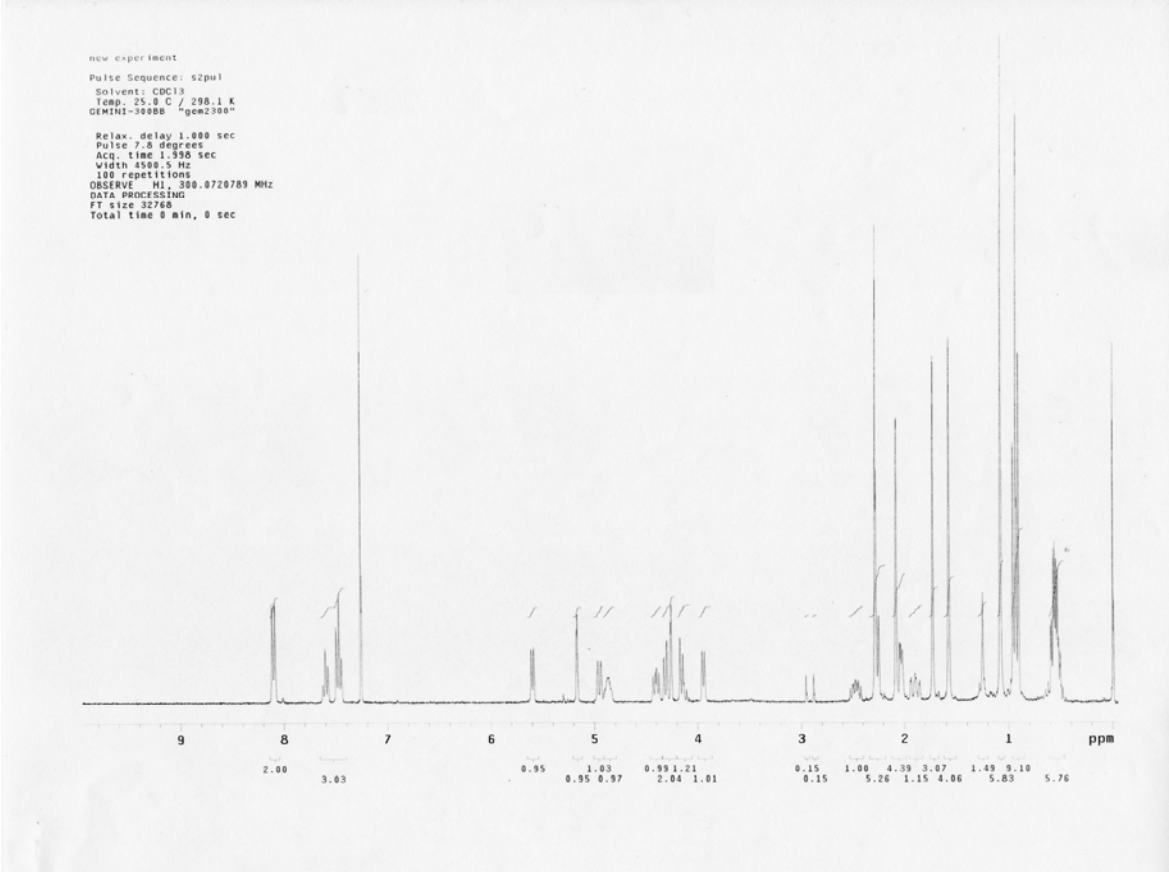
1H NMR Spectrum of **1-*t*-Boc-(3*R*)-(TIPS-O)-(4*S*)-difluorovinylazetidin-2-one (2-10)**



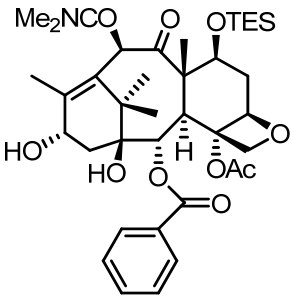


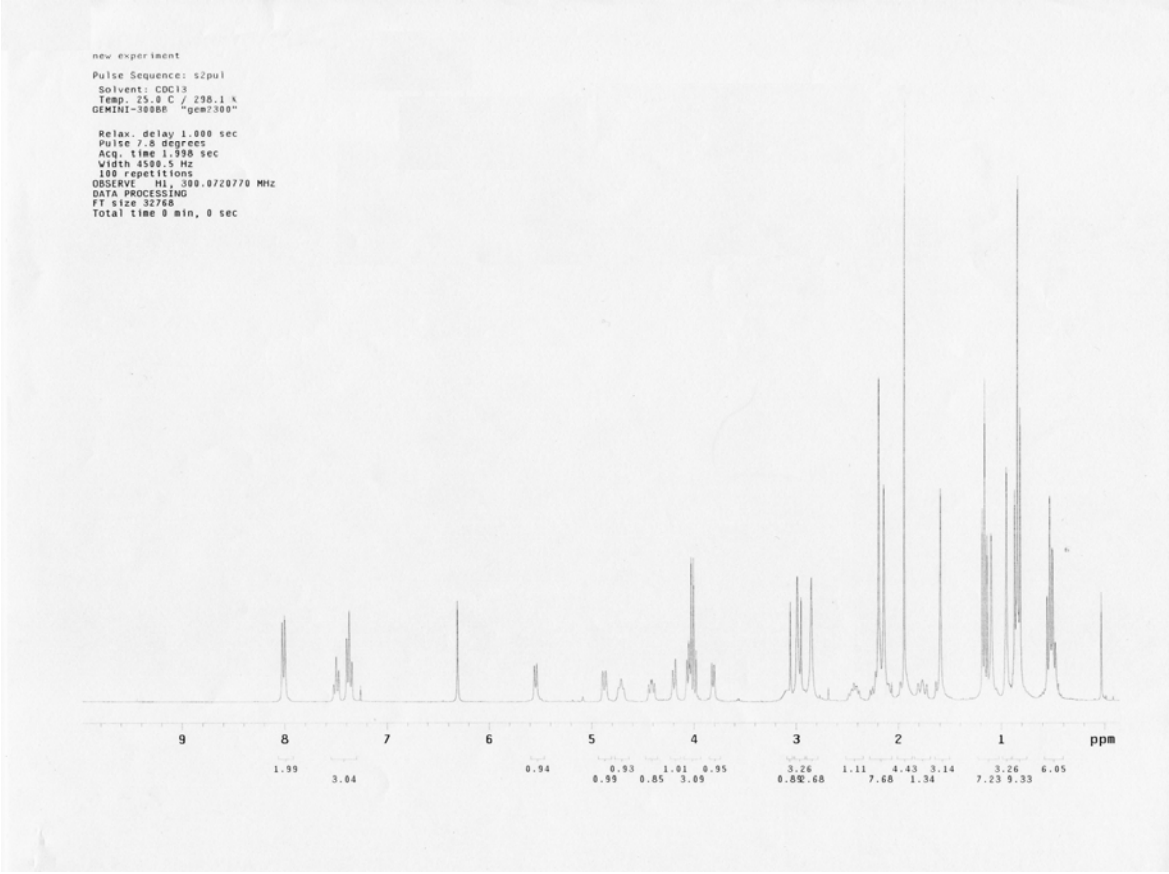
^{1}H NMR Spectrum of **7-Triethylsilyl-10-deacetylbaccatin III (3-2)**



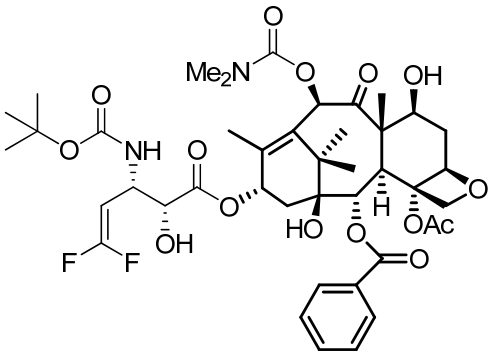


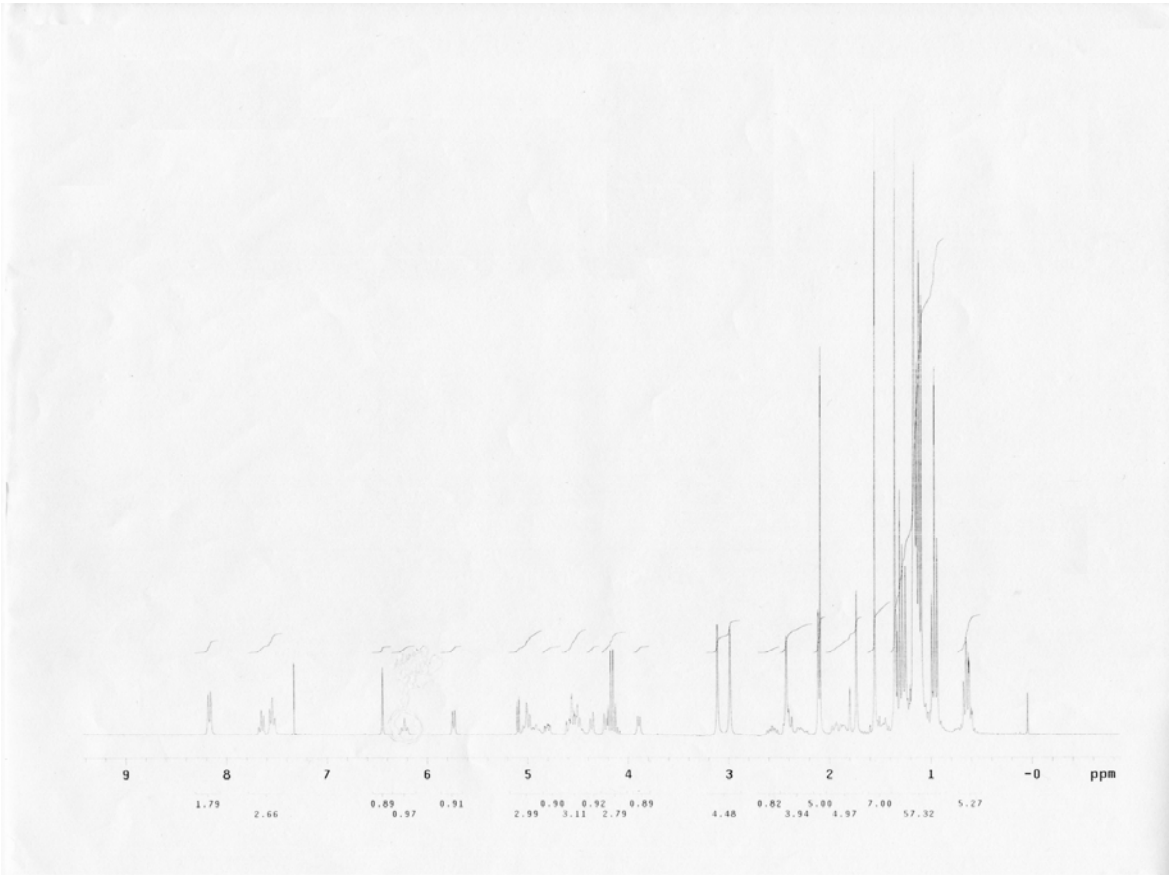
^{1}H NMR Spectrum of **7-Triethylsilyl-10-deacetyl-10-dimethylcarbamoylbaccatin III (3-3)**





^{1}H NMR Spectrum of **3-Dephenyl-3-(2,2-difluorovinyl)-10-dimethylcarbamoyldocetaxel (3-5, SB-T-12854)**





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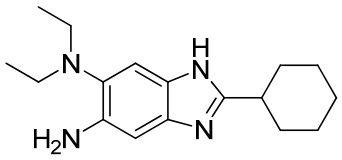
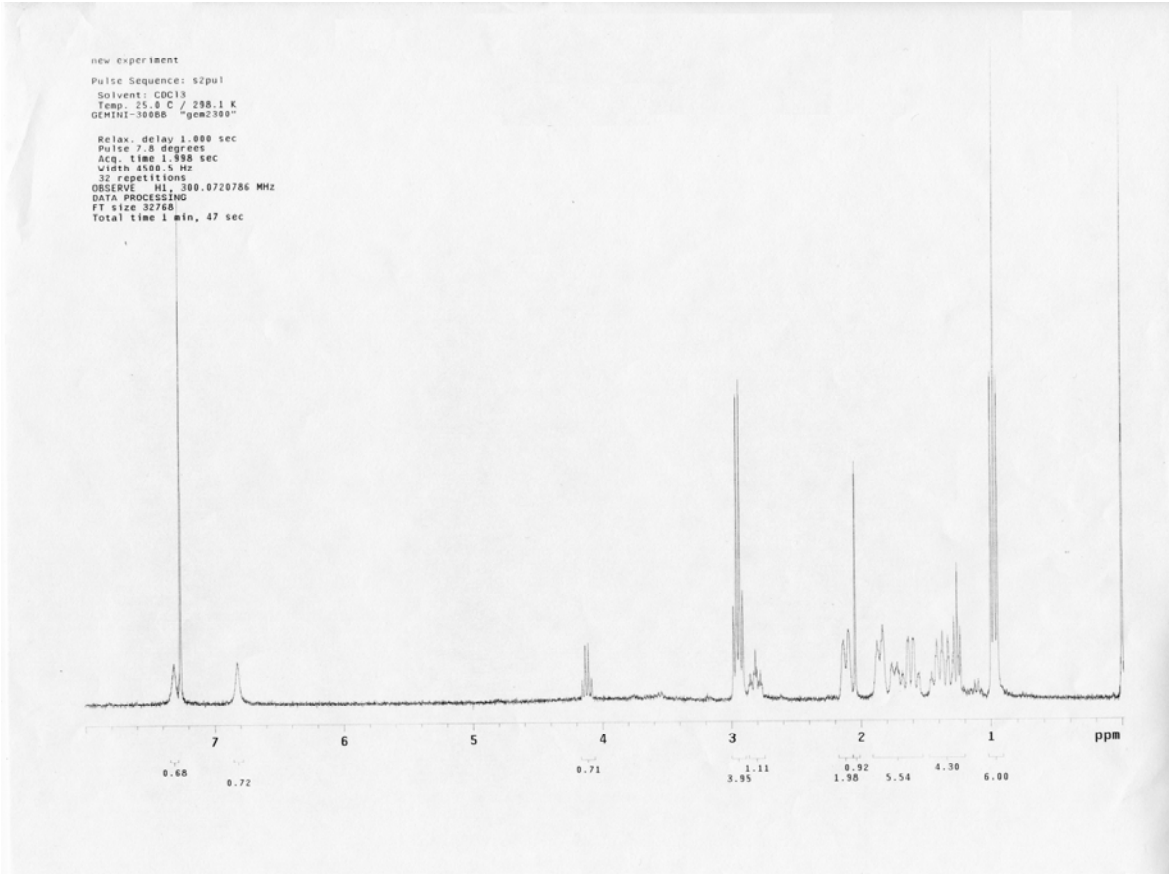
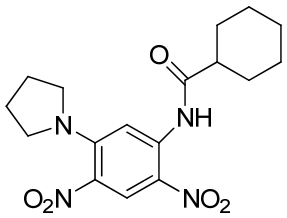
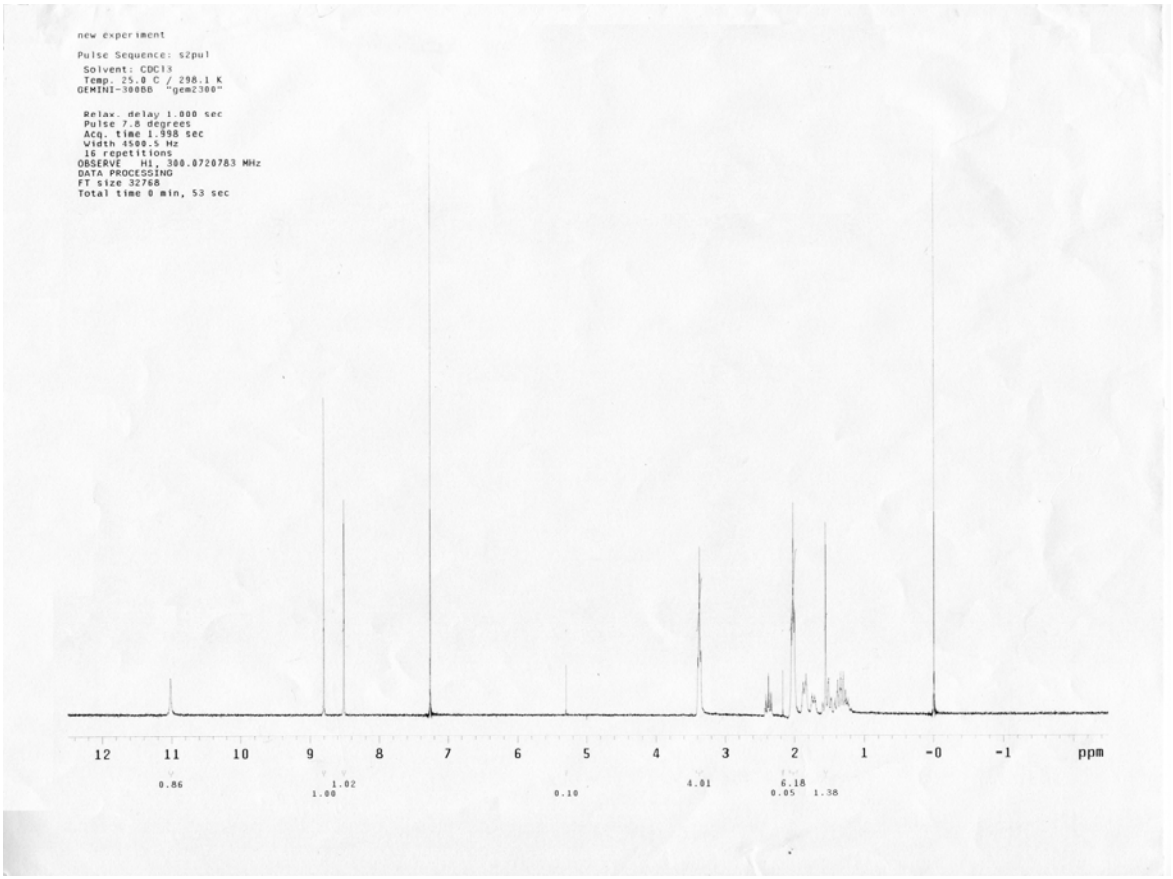


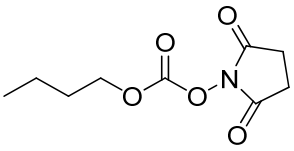


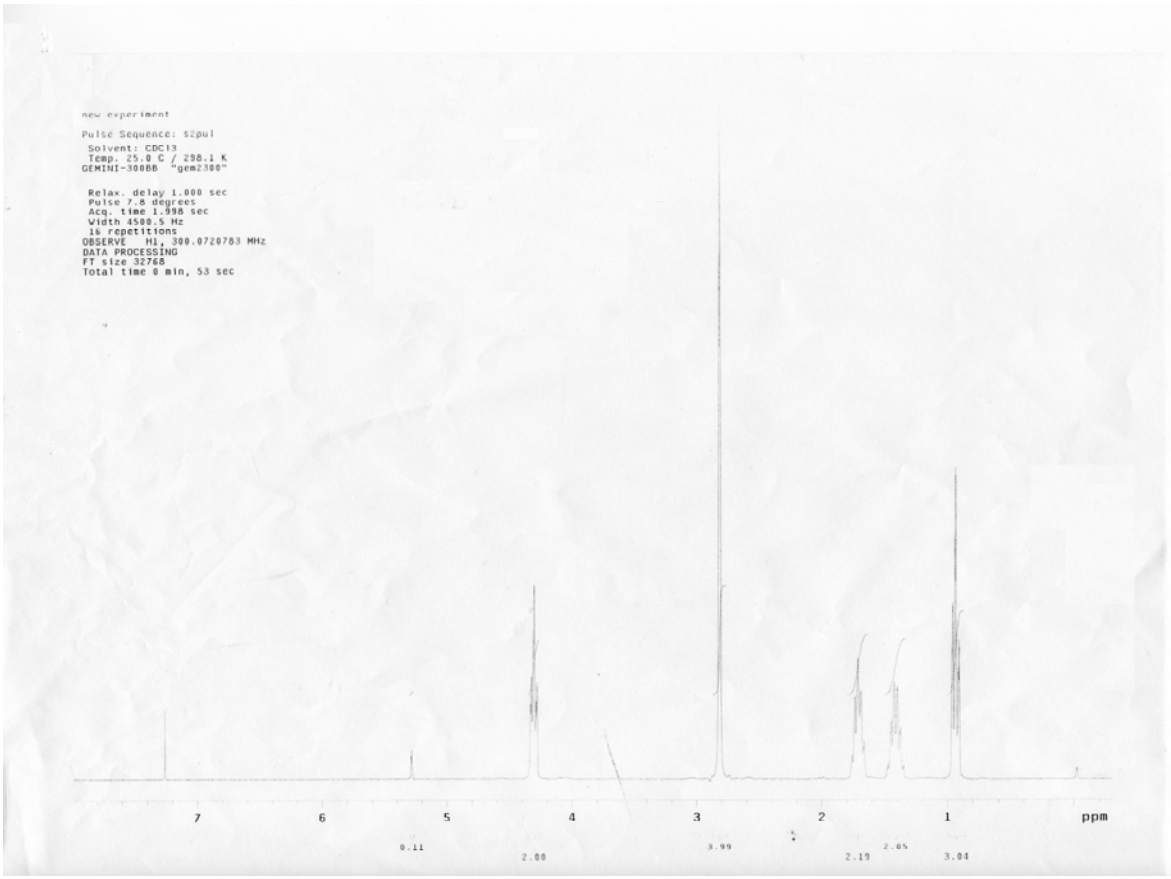
^{1}H NMR Spectrum of ***N*-2,4-Dinitro-5-(pyrrolidin-l-yl)phenyl cyclohexanecarboxamide (4-2d)**





^{1}H NMR Spectrum of **2,5-dioxopyrrolidinyl-n-butylcarbonate (4-5)**





^{1}H NMR Spectrum of **2-cyclohexyl-5-butoxycarbonylamino-6-pyrrolidinylbenzimidazole (4-6)**